



DISSERTATION

The Effects of Probiotic and Conventional Yoghurt on Lipid Profile and Parameters of Oxidative Stress in Healthy Women

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LIST OF ABBREVIATIONS

ACAT	Acyl CoA Cholesteryl Acyl Transferase
ABTS	2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate)
ANOVA	Analysis of Variance
Apo A	Apolipoprotein A
Apo B	Apolipoprotein B
ATP	Adenosine Triphosphate
BMI	Body Mass Index
CD	Conjugated Dienes
CFU	Colony Forming Units
CHOD-PAP	Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase
CLA	Conjugated Linoleic Acid
CoA	Coenzyme A
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic Acid
DPPH	a,a-diphenyl-b-picrylhydrazyl
DVS	Direct Vet Set
FM	Fermented Milk
EPS	Extracellular Polysaccharides
GALT	Gut-associated Lymphoid Tissue
GI	Gastro Intestinal
GPO-PAP	Glycerol Phosphate Oxidase Phenol 4-Aminoantipyrine Peroxidase
GSSG	Glutathione Disulfide
GSH	Glutathione
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HMG CoA	Hydroxy Methyl Glutarate CoA
HMGR	Hydroxy Methyl Glutarate Reductase
HSDH	Hydroxy Steroid Dehydrogenase
IBD	Inflammatory Bowel Disease
IDL	Intermediate Density Lipoprotein
IPP	Isopentenyl Pyrophosphate
IQR	Inter Quartile Range
IL	Interleukin
IgG	Immunoglobulin G
LAB	Lactic Acid Bacteria
LCAT	Lecithin-Cholesterol Acyl Transferase
LDL	Low Density Lipoprotein
LPL	Low Density Lipid
MDA	Malondialdehyde
MDP	Muramyl Dipeptide
MUFA	Monounsaturated Fatty Acid
NF- κ B	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
NK	Natural Killer
4NQO	4-nitroquinoline-N-oxide
RDA	Recommended Dietary Allowances
ROS	Reactive Oxygen Species
PUFA	Polyunsaturated Fatty Acids
SCFA	Short Chain Fatty Acids

SD	Standard Deviation
SFA	Saturated Fatty Acids
SIgA	Secretory Immunoglobulin A
SOD	Superoxide Dismutase
STZ	Streptozotocin
TAA	Total Antioxidative Activity
TAC	Total Antioxidant Capacity
TAS	Total Antioxidative Status
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TNF- α	Tumor Necrosis Factor-alpha
USDA	United States Department of Agriculture
VLDL	Very Low Density Lipoprotein

1 INTRODUCTION

Cardiovascular disease (CVD) is considered a multifactorial disease. Its mortality rates are high and in several countries it is known to be one of the most dominant causes of death. Widespread studies, both clinical and epidemiological, have shown that increase in the risk of coronary heart disease and heart attack can be attributed to several factors. Abnormal level of blood lipid is one of the major risk factors of CVD. There are a number of studies that indicate oxidative stress has a role in initiating numerous diseases, including cardiovascular disease, hypercholesterolemia, atherosclerosis, and hypertension, (Touyz and Schiffrin, 2004; Paravicini and Touyz, 2008; Shah et al, 2008; Harrison and Gongora, 2009).

There are some evidence that probiotics may reduce the risk of cardiovascular diseases in addition to their effect on gastrointestinal disorders and other health benefits. Probiotic bacteria have been the subject of interest in both the scientific and the commercial world. The reason for this interest lies in the assortment of health effects of these particular types of bacteria on humans. Probiotics, which are living microorganisms, could help in wellbeing of humans, if consumed in appropriate quantity. Probiotic microorganisms can be found as components of foods, dairy products and in the form of supplements or beverages. These bacteria and yeasts have been used for many years for fermenting foods. Such useful bacteria, especially specific strains of *bifidobacteria* and *lactobacilli*, can be found in specific food such as yoghurt and milk based products.

Lactobacilli and Bifidobacteria that comprise a chief part of the normal intestinal microflora in animals and humans are Gram-positive lactic acid-producing bacteria. The gastronomical environment and the microflora that live in it play a significant role in maintaining the health of humans. The microflora is comprised of all sorts of bacteria that could be potentially useful or damaging to human health. It is, therefore, important to maintain a healthy intestinal tract that allows absorption of nutrients, while keeping out toxins and pathogens. By consuming probiotics, specifically certain species of *bifidobacteria* and *lactobacilli*, the balance of flora can be assisted. This balance is created through increasing the number of helpful and reducing harmful bacteria in the intestine. Consumption of probiotics can also enhance the immune response of the gut and improve its function as an important barrier against harmful bacteria.

The most conclusive evidence offered for the benefits of probiotics is, probably, their anti-diarrheal and stool regulating effects (Gionchetti et al, 2000; Levy, 2000; Benchimol and Mack, 2004; de Vrese and Marteau, 2007). There is significant evidence that eating fermented milk with adequate amounts of live and active yoghurt cultures can reduce symptoms of lactose intolerance in humans (Guarner et al, 2005; Parvez et al, 2006; de Vrese and Schrezenmeir, 2008). Studies have shown that individuals suffering from rotavirus or travelers' diarrhea could benefit from probiotics which can reduce its severity and the length (Solga, 2003; Tomas et al, 2004; Johnston et al, 2007). Certain strains of probiotics have shown immunomodulatory effect. They can improve immune defenses of the mucosal cells in the intestine through both non-specific and specific effects. Some evidence show that certain strains have positive effects on allergy (Kalliomaki and Isolauri, 2004; Isolauri and Salminen, 2008). A growing number of studies show that probiotics may have positive effect on blood lipid level, as well (Gilliland et al, 1985; Agerholm-Larsen et al, 2000; Tamime, 2002; Parvez et al, 2005; Fabian and Elmadfa, 2006).

Probiotics and hypocholesterolemia

Cholesterol is essential in functioning of the body. It is necessary for formation of certain hormones and vitamins, and is an essential component of cell membrane and nerve cells. However, high levels of cholesterol or some other lipids could lead to cardiovascular disease. While in our body cholesterol is made to keep the required level for different functions, our diet also influences the plasma lipids. Many studies have investigated the effect of lactic acid bacteria on lipid profile.

One of the first studies on the effect of fermented milk on cholesterol concentration was conducted by Mann and Spoerry (Mann and Spoerry, 1974) in Maasai tribes. This initiated more studies to investigate the possible relation between cholesterol and fermented milk.

Several in vitro studies have shown that some strains of LABS such as *bifidobacteria* and *lactobacilli*, in the presence of bile acids, are capable to assimilate cholesterol.

Probiotic bacteria are able to produce short-chain fatty acids (SCFA) through fermenting carbohydrate in food. Production of the SCFA in the gut, could block synthesis of hepatic cholesterol or redirect plasma cholesterol toward the liver, and as a result lower the plasma cholesterol. Additionally, some strains are able to block cholesterol absorption through

deconjugating bile salt, or disrupting cholesterol mechanism through assimilating it directly, in the gut.

Evidence from some animal studies, proposes that some fermented milk products are capable to reduce cholesterol moderately. However, studies on human have not been decisive on their effect on reducing cholesterol or low density lipoprotein (Lewis and Burmeister, 2005 Greany et al, 2008; Klein et al, 2008). The inconsistency in results is probably due to differences in the bacteria strains, designs of the studies, and confounding factors such as intake of fermented milk, which make it difficult to draw a conclusion.

Probiotics and oxidative stress

Human body posses an antioxidant mechanism for defense as well as a repair system. This system which has been developed to defend the body against oxidation destruction is not sufficient to avoid damage. However, those foods or supplements which have antioxidant properties could be beneficial in decreasing the oxidative injury.

Oxidative stress takes place when the creation of reactive oxygen species (ROS) in a system surpasses its ability to neutralize and eradicate them. ROS encompasses non-radicals and radicals derived species. These molecules have unpaired electrons and usually are very reactive. They are produced in the cell constantly as a result of cell respiration or as metabolism by-products. Molecular oxygen and hydroxyl radicals or superoxide anion, which are its radical derivatives, in addition to peroxides and transition metals are among main free radicals in aerobic cells (Percival, 1998).

A highly complex antioxidant protection system operates in the human body to block creation of free radicals. The system has different components such as proteins to seize transition metals, enzymes to stop peroxides, and also a mechanism to hunt ROS. When biological molecules in the body get oxidized by ROS, disease and tissue damage occur.

Studies on the antioxidant properties of lactobacillus have been conducted recently There are studies on the antioxidative ability of some strains of bifidobacteria and lactobacilli (Kullisar et al, 2002; Lin and Chang, 2000). Antioxidant activity shown by lactic acid bacteria in their intracellular cell-free extract are through different mechanisms including;

hunting ROS, chelating ability for metal ion, blocking enzymes activity, and reducing ascorbate autoxidation (Lin and Yen, 1999).

The ability of starter cultures to hunt free radicals in fermented milks and other dairy products could offer another supply of antioxidants in the body, by giving a chance to probiotics to make antioxidant while they are increasing in the GI tract (Ouwehand and Salminen, 2002). Depending on the culture applied in each study, the power of antioxidants varies.

The evidence about the effect of probiotic yoghurt on lipid profile and oxidative stress parameters are not conclusive. Furthermore, in the studies that were conducted, probiotic and conventional yoghurt consuming groups were compared. In the present study in addition to the two yoghurt groups a third group which did not use fermented products was considered. The aim of the present study is to compare the effect of probiotics yoghurt containing *Lactobacillus acidophilus* LA5 and *Bifidobacteria lactis* BB12 and conventional yoghurt on the lipid profile and parameters of oxidative stress in healthy women.

2 GOAL AND OBJECTIVES

2-1 Goal

The main goal of this study is to compare the effects of consumption of probiotic yoghurt containing *Lactobacillus acidophilus* LA5 and *Bifidobacteria lactis* BB12, conventional yoghurt and not fermented dairy products in the diet, on lipid profile and parameters of oxidative stress in healthy women.

2-2 Analytical objectives

Related to Lipid profile:

1- To compare the mean concentrations of lipid profile, including total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, total to HDL cholesterol ratio, Apo A, and Apo B at the baseline among three groups: groups consuming conventional yoghurt, probiotic yoghurt and control group (consuming no fermented dairy products).

2- To compare the mean concentrations of lipid profile within each group at each interval, at baseline, after 3 weeks and after 6 weeks.

3- To compare the mean differences between baseline and 6th week concentrations of lipid profile among the three groups.

4- To assess the correlation between the mean differences in total to HDL cholesterol ratio and total cholesterol on the one hand, and HDL cholesterol on the other, for each group.

Related to potentially oxidant and antioxidant parameters:

1- To compare the mean concentrations of malondialdehyde oxidized LDL, and total antioxidant capacity at baseline, between the three groups: conventional yoghurt, probiotic yoghurt, and control.

2- To compare the mean concentrations of malondialdehyde, oxidized LDL, and total antioxidant capacity, within each group at each interval (at baseline, after 3 weeks and after 6 weeks).

3- To compare the mean differences between baseline and 6th week concentration of malondialdehyde oxidized LDL, and total antioxidant capacity among the three groups.

Related to dietary intake parameters:

1- To compare the intakes of calorie, fat, cholesterol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, dietary fiber, beta carotene, vitamin C, and E at baseline among the three groups; conventional yoghurt, probiotic yoghurt, and control.

2- To compare the intakes of calorie, fat, cholesterol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, dietary fiber, beta carotene, vitamin C, and E within each group at each interval (at baseline, after 3 weeks and after 6 weeks).

3- To compare the mean differences between baseline and 6th week values of calorie, fat, cholesterol, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, dietary fiber, beta carotene, vitamin C, and E among the three groups.

Individual related parameters:

1- To compare the body weight and body mass index at baseline among the three groups: probiotic yoghurt, conventional yoghurt, and control.

2- To compare the body weight and body mass index within each group at each interval.

Hypothesis:

- 1- There are improvements in lipid profile parameters in probiotic and conventional yoghurt consuming groups compared to the control group.
- 2- There is a decrease in malondialdehyde and oxidized LDL concentration and increase in total antioxidant capacity in the probiotic and conventional yoghurt consuming groups compared to the control group.

3 LITERATURE REVIEW

3-1 Probiotics

3-1-1 Probiotic definition

The term probiotics denotes “for life” and refers to microorganisms which have positive effects on human health. Micro-organisms presiding in food, especially lactic acid bacteria were believed to have beneficial health advantages from long ago. Abraham’s longevity, as asserted in the Old Testament (Persian edition), was due to the sour milk he drank. The Roman historian, Plinius, treated gastroenteritis with the help of milk products that were fermented. This dates back to 76 BC (Schrezenmeir and *de Vrese*, 2001).

Since the introduction of the science of microbiology, researchers, including Tisser and Metchnikoff, have related these benefits to changes in microbial balance of the intestine (Tisser, 1984). The father of modern immunology, Metchnikoff, suggested that the consumption of milk fermented with *Lactobacillus delbrueckii* caused the evident long life of the Balkan peasants. His argument was that these bacteria would grow faster than the other damaging bacteria and hence reduce or inhibit their harmful activity in the gastro intestinal tract. This, in turn, would enhance our health by decreasing the amount of toxins in the gut. Tisser believed that diarrhea in infants could be treated with *bifidobacteria*. He suggested that these bacteria would render the putrefactive bacteria, that cause diarrhea, ineffective (Sanders, 2003).

The term “probiotics” was created around the 1960’s, but the concept had been around from the beginning of the 20th century. As years have gone by the meaning of this term has changed. However the definition given by the Joint Food and Agriculture Organization/World Health Organization Working Group in 2001, i.e.; “*Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host*” seems to be the most accurate.

The probiotic bacteria selected should have the following main characteristics: (Krishnakumar, 20001)

- They should have “non-pathogenic” activity
- They should be “resistant to bile salts” and gastric acids
- They should have the “desired technological and organoleptic properties”

- They should have “biological efficiency” on humans, including ‘adhesion to epithelial cells’ in the intestine
- They should interact with “enteropathogenic” bacteria
- They should be able to “colonize” in the gut
- They should be able to “stimulate the immune system”.

3-1-2 Important aspects of probiotics

Viability

On the definition of the term, even though many scientists insist on the viability of the microorganisms used as probiotics, several studies have shown that non-viable probiotics, too, can attach to the intestinal wall and reduce duration of diarrheal diseases (Conconier et al, 1993; Guarner et al, 2005). Studies examining probiotics’ immunological attributes have indicated that when living cells were given to patients, it led to a considerable increase in the number of cells that secrete IgA. The viability of the probiotic cells is essential for the probiotic antigens to be taken up through the Peyer’s patch, and also to adhere to the M-cells within the intestine (Isolauri, 1999; Guandalini et al, 2000; Szajewska et al, 2007). Halpern et al (Halpern et al, 1991) showed in their study that consumption of yoghurt with living cells resulted in T-cells producing higher amounts of gamma-interferon.

Survival within the GI tract

In order for probiotics to have beneficial health effects, they must be able to travel through the upper gastro-intestinal (GI) tract intact. They must pass through the GI safely so as to reach the location they are to colonize. In order for the colonization to happen, probiotic must adhere to intestine’s epithelial and mucosal cells. This affects the length of time the bacteria stays within the intestine. Also, it has an impact on the functional performance of the bacteria. The bacteria are strained by a number of stress factors while moving forward in the intestine. One such factor is the extremely low acidity (pH 1.5-3) of the stomach. The lowest acidity in which LAB can survive is 3.0, considerably higher than the acidity in stomach (Olejnik et al, 2005).

The extremely high pH environment in the stomach is a crucial parameter in choosing LAB strains. This harsh condition has a powerful impact on the viability of bacteria in the stomach. Olejnik et al. and Lee and Wong demonstrated that yoghurt’s pH reduced viable L.

casei cells, for 13 days, by 2 log-cycles, and when the pH reached 6.5, more than 30 days. These figures were 3 and 30 days at pH 4 and 6.6, respectively, for *L.acidophilus* CH5. Furthermore, *B. bifida* which is vulnerable to acidity, viable cells reduced after 4 and 15 days at pH4.3 and 6.6. Bacteria that are entered in the stomach stay there for 1 to 4 hours at pH less than 3.0 (Olejnik, 2005; Lee and Wong, 1998). That is long enough to kill most of bacteria.

Pepsin is the next important parameter that has an impact on the bacteria. Various proteins that are involved in adhesion and aggregation activities, like binding mucus and collagen, are in the bacterial cell walls. It is able to hydrolyze these proteins. This can have an impact on adhesive characteristics of bacteria and the integrity of cytoplasm membrane. Other lipolytic and proteolytic enzymes produced in small intestine can adversely affect survival of the bacteria. Conjugated bile salts, too, have a devastating effect on the bacteria. They can ruin plasmatic membrane's structure through emulsification. These greatly reduce the adhesive properties and the viability of the bacteria as they move through the gastrointestinal tract.

Human origin

There is disagreement on whether the probiotics used should be of human origin. But, as long as it can be demonstrated that they can stay alive while passing through the GI tract, especially through the harsh stomach environment with induction of new genes encoding a number of stress proteins, and as long as they can show to be able to colonize in the large intestine, this is not a matter for concern. (Ljungh and Wadström, 2006)

Adhesion

It has been suggested that probiotic bacteria's attachment to the intestine's wall and their colonization of the gastro-intestinal tract is a vital precondition for their survival and functioning. Strains of probiotic bacteria with adherent properties are likely to stay longer in the intestine. They, thus, have better chances for manifesting immunological and metabolic effects, compared to the strains with no adhesion properties. The bacteria's adhesion property causes them to interact with the mucosal surface and makes contact with gut associated lymphoid tissue (GALT) possible. It also plays a role in intestinal and systemic immune effects. Only probiotics with adherent properties are believed to stabilize the

intestinal mucosal barrier and to be instrumental in inducing the immune effects. Adhesion may also administer routes of exclusion of harmful bacteria and keep them away from the epithelium. Various strains of probiotic bacteria possess different adhesive properties.

It could be suggested that powerful capability for adhesion may raise the risk of infection in the individual. Certain strains of probiotics which are poorly adhering in vivo or in vitro still can show positive effects in the hosts (Saarela et al, 2000).

Safety

Both public health officials and scientific authorities seek assurances that a probiotic product introduced to the market can deliver live strains of the bacteria in sufficient numbers to the large intestine so that it may provide the desired benefit for the consumers.

The amount of probiotics found in different food products differs a great deal. It has been suggested that in order for probiotic bacteria to have their therapeutic effects, at least 10^8 colony forming units (CFU) should be consumed daily (Lourens-Hattingh and Viljoen, 2001). These elevated amounts are necessary, it has been argued, because it should make up for the probable decrease in the probiotic organisms as they pass through the GI tract. The growing public interest in dairy products containing probiotics has induced a number of food companies to introduce minimum standards for the concentration of probiotic strains in their products. These require a minimum of 10^6 - 10^7 cfu/ g⁻ of *L. acidophilus* and/or *Bifidobacteria* in their dairy products (IDF, 1992; Shah, 2000). It is further recommended that at least 10^6 cfu/g of probiotic bacteria present in probiotic yoghurts at the time of sale (Kurmann and Rasic, 1991).

Based on epidemiological data on the effect of probiotics on the safety of dairy products, there is no evidence that these microorganisms are involved with human infections (Saavedra et al, 2000; Rolfe, 2000). Nevertheless the likelihood of infections resulting from the use of probiotics remains, infections that cause different reactions to a specific strain in different individuals. There are reports on infections induced by lactic acid bacteria. The majority of cases, however, concern patients with serious dysfunctional immune systems. One should also remember that during the last twenty years the taxonomy of several LABs has been rebuilt (Ouwehand et al, 2002). The application of modern multi-phased classification has resulted in the re-categorization of many probiotic strains. Over all, probiotics are deemed quite harmless. Numerous products containing probiotics have been used conventionally over centuries and their safety has been verified.

Resistance to antibiotic is increasingly a common characteristic among micro-organisms. This is a result of widespread and unchecked consumption of antibiotics in both human and animals. This has resulted in great difficulties in treating and healing of infections. Resistance of bacteria to antibiotics can be inherent or it may be acquired. Inherent resistance can be looked at as a species trait and is an innately occurring property. Acquired resistance to antibiotics, however, is caused by genetic alterations. Getting alien DNA from other bacteria can also cause this resistance.

Antibiotic resistance (by plasmid-linked), is unusual in *lactobacilli*. Nevertheless it does take place and its consequences as far as the safety of humans is concerned need to be noted. As transfer of genes that are resistant to antibiotics may occur between bacteria which are phylogenetically unrelated, specific strains that carry the transportable components that have resistance genes should not be utilized. This holds true for both human products as well as animals. In short, safety issues concerning probiotics cover the following attributes: (Saarela et al, 2000)

1. Strains that have human origin are preferred over those with non-human origin
2. They should be isolated from healthy human GI-tract.
3. They should be non-pathogenic.
4. They should not have a history of association with such diseases as GI-disorders or infective endocarditis.
5. They should not deconjugate bile salts (this would be a negative trait in the small bowel).
6. They should not convey antibiotic resistance genes which can be transmitted.

Technological aspects of probiotics

It is necessary that probiotic foods have good sensory properties and that they be safe to consume. They should also contain specific probiotic strains at an adequate level through the storage. It must be possible to produce probiotic strains in industrial settings without losing their functionality during storage as cultures in frozen or freeze dried form (Saarela, 2000). Only then can they be given to customers. Furthermore, probiotic strains should not produce off-flavours or textures in the foods into which they are incorporated. In order to ensure the quality of probiotic bacteria, the resources used for packaging and also the circumstances the products are stocked and stored assume great importance.

Most commercial cultures for probiotic products come in highly concentrated form, and are meant for DVS (direct vet set) application. It is customary to utilize these afore-mentioned highly concentrated direct vet set cultures because of the challenges in production of micro-organism in the location that manufacturing takes place. The DVS cultures usually come in two forms; freeze-dried cultures or highly concentrated frozen cultures.

3-2 Lactic acid bacteria as probiotics

It has been suggested that a number of *Bifidobacterium* sp, *Lactobacillus* species, *Saccharomyces* boulardii, and several other microbes be utilize as probiotics. They are usually, chosen from bacteria that normally live in the human gastrointestinal system and cause no health risk. These bacteria are first purified. Then they are cultivated and concentrated in high dosages and preserved. Basically, they are provided in the following forms (Saarela, 2000):

- They are added as a “condense culture” to milk products or food, at medium level, with a very low chance, or no chance at all for culture growth.
- They are added to “milk-based product” or supplement through inoculation and grow until they reach adequate levels in a fermented food.
- They are provided as “condensed and dried cells packaged” in form of dietary supplements such as tablets, capsules, and powders.

As probiotics, lactic acid bacteria have been associated with milk products for a long time. The reason is that a number of the same strains which are found in dairy products also reside in different parts of our body, such as the vagina, the mouth, the stomach, and the gastrointestinal tract. Some of these bacteria, therefore, can be used as a vehicle for transforming milk into a variety of dairy products which are fermented (cheese, yoghurt, kefir, etc.). They can also play the additional role of contributing to the process of colonizing bacteria. Fermented milk products can, therefore, be a suitable medium to provide probiotic.

Probiotic bacteria are not identical. They are different in their gene make up, species and strain. Strains of the same species may differ in traits and attributes. Such attributes consist of type and extent of inhibitors they create, stability, manifestation of enzymes, forms of carbohydrate fermentation, their ability to produce acids, their non acceptance of bile and

acids, their capability to inhibit the GI tract. Also perhaps the most significant attribute is the strains proven clinical ability to produce desired effects (Sanders, 2000).

Various yeasts and bacteria used as probiotics, including:

- *Lactobacillus*
- *Bifidobacterium*
- *Lactococcus*
- *Saccharomyces* (yeast)
- *Streptococcus Thermophilus*
- *Enterococcus*

3-2-1 *Lactobacillus*

Lactobacillus is gram-positive bacteria. They are able to function in the presence or absent of oxygen. *Lactobacilli* are an important part of the LAB bacteria. They are called by this name because the majority of them are able to change lactose and other simple carbohydrate to lactic acid. *Lactobacilli* normally live in the human intestine and vagina. They are usually live in vagina or GI tract in the human body. They are considered as a major species of the gut flora (Olejnik et al, 2005). Some *Lactobacilli* used as probiotics include:

<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i> BG2FO4
<i>Lactobacillus casei</i>	<i>Lactobacillus johnsonii</i> LA1
<i>Lactobacillus crispatus</i>	<i>Lactobacillus acidophilus</i> NCFM
<i>Lactobacillus bulgaricus</i>	<i>Lactobacillus acidophilus</i> INT-9
<i>Lactobacillus curvatus</i>	<i>Lactobacillus plantarum</i> ST31
<i>Lactobacillus brevis</i>	<i>Lactobacillus reuteri</i>
<i>Lactobacillus plantarum</i>	<i>Lactobacillus casei</i> Shirota
<i>Lactobacillus rhamnosus</i>	<i>Lactobacillus acidophilus</i> DDS-1
<i>Lactobacillus GG</i>	<i>Lactobacillus acidophilus</i> NCFB 1748
<i>Lactobacillus cellobiosus</i>	<i>Lactobacillus delbrueckii</i>
<i>Lactobacillus fermentum</i>	<i>Lactobacillus salivarius</i> UCC 118
<i>Lactobacillus gasseri</i>	<i>Lactobacillus acidophilus</i> SBT-2062
<i>Lactobacillus johnsonii</i>	<i>Lactobacillus paracasei</i>
<i>Lactobacillus salivarius</i>	

3-2-2 Bifidobacterium

Bifidobacteria normally live in the human and animal GI tract. In less than a few days after birth, infants are colonized with these bacteria for the first time *Bifidobacteria* was isolated from breast-fed baby's feces. They are gram positive and usually have a Y form. The number of these bacteria is relatively constant in early stage of life, but later it decreases as a result of drugs such as antibiotics, and because of changes in the diet. They are able to generate lactic acid without making carbon dioxide. They are also categorized as LAB bacteria. Some *Bifidobacteria* applied as probiotics include:

<i>Bifidobacterium adolescentis</i>	
<i>Bifidobacterium infantis</i>	<i>Bifidobacterium longum</i> BB536
<i>Bifidobacterium breve</i>	<i>Bifidobacterium infantis</i> RO33
<i>Bifidobacterium animalis</i>	<i>Bifidobacterium breve</i> RO70
<i>Bifidobacterium longum</i>	<i>Bifidobacterium bifidum</i> RO71
<i>Bifidobacterium bifidum</i>	<i>Bifidobacterium lactis</i> Bb12
<i>Bifidobacterium thermophilum</i>	<i>Bifidobacterium longum</i> RO23
<i>Bifidobacterium lactis</i>	<i>Bifidobacterium longum</i> SBT-2928
<i>Bifidobacterium breve</i>	

3-2-3 Lactococcus

Lactococci are also gram-positive bacteria which can function with or without oxygen. Also they are considered as part of the family of lactic acid bacteria. *Lactococcus lactis* (or *streptococcus lactis*) can be found in milk-based products. Souring of milk is a result of *Lactococci* bacteria activity. *Lactococci* that are used as probiotics include:

Lactococcus lactis, subspecies *cremoris*

Lactococcus lactis, subspecies *lactis* NIAI 1061

Lactococcus lactis, subspecies *lactis* NIAI 527

Lactococcus lactis, subspecies *lactis* NCDO 712

Lactococcus lactis, subspecies *lactis* biovar *diacetylactis* ATCC 13675

Lactococcus lactis, subspecies *lactis* biovar *diacetylactis* NIAI 8 W

3-2-4 Saccharomyces

Saccharomyces is categorized as yeast. *Saccharomyces boulardii* is the main probiotic yeast. *S. boulardii* is usually not pathogenic yeast. It is administered in treatment of antibiotic induced diarrhea.

3-2-5 Streptococcus Thermophilus

Streptococcus thermophilus, is a gram-positive bacterium that is able to function anaerobically and aerobically. It is a catalase-negative, homofermentative, and non-motile bacteria. It, too, is considered as a member of lactic acid bacteria family. *Streptococcus thermophilus* can be found in milk and milk-based products. It is one of the main strains needed for production of yoghurt. Another probiotic strain is *Streptococcus salivarius subspecies thermophilus* type 1131.

3-2-6 Enterococcus

Enterococci are also among gram-positive bacteria. They are facultative anaerobics so can function anaerobically and aerobically and are catalase-negative. They are generally not motile, and non-spore forming. *Enterococci* reside in the intestine and are part of the normal flora of humans and animals. A probiotic strain of these bacteria is *Enterococcus faecium* SF68, which has shown to be effective in diarrheal diseases.

3-3 Probiotics - mechanisms of action

Probiotic bacteria benefit human being in a variety of manners. Releasing antimicrobial substances in the body, resisting colonization of harmful bacteria, stopping pathogens from

attaching to GI tract, breaking up destructive elements, enhancing the immune system, boosting the activity of brush border enzymes, are among probiotics' actions that affect our health and wellbeing (Isolauri et al, 2001; Walker, 2000; Ruemmele et al, 2009; Isolauri and Salminen, 2008; Salminen et al, 2006; Zhang et al, 2005).

Most probiotic agents can generate compound with antimicrobial properties (Organic acids, hydrogen peroxide, and bacteriocins) that are able to decrease pathogens in a direct manner or work indirectly by changing their metabolism and prevent endotoxin activity (Ingrassia et al, 2005; Candela, 2008). In order to understand how probiotic bacteria operate, one has to have a good insight of the microbiology and physiology of the gastrointestinal tract.

3-3-1 The gut microflora

The human gastrointestinal tract is eight meters long and has a surface area the size of a football field. From amongst all the organs in the body the GI tract has the most interaction with the environment. Thus, its role is to ingest and absorb nutrients. It is also in charge of the emission of waste products. As the largest organ that is involved in the functioning of our immune system, it creates 80% of the antibody in our body. Of the 100 billion bacterial cells (1 kg bacteria) within the intestinal microflora, many are useful while others can be harmful. Some are capable of being both, while the function of others is uncertain.

Human GI tract is sterile until birth, when microflora are introduced to it through mother's vagina and feces. The colon population in babies that take milk directly from mother's breast consists of 90% *bifidobacteria*, as well as certain amounts of *enterobacteriaceae* and *enterococci*. However, there are almost no *staphylococci*, *bacteroides*, *clostridia*, or *lactobacilli present*. These microfloras are important for formation and development of the immune function of the gut, both in the post-natal period and also for its development in the first years after birth. From approximately the age of three the composition of the flora becomes extremely complicated. This will last all the way to the age of maturity. The equilibrium of the microflora plays a vital role in the preservation of the intestine and definitely in preserving the body throughout life. The bacteria colony varies depending on the location. This is because of the various environments within the tract. The number and type of the indigenous microflora varies in different parts of the GI tract (Gionchetti et al, 2000). For example, since the acidity level is not suitable for bacteria, there is only minor

colonization in the stomach. This is also the case for the upper section of the small intestine. In the lower section of the small intestine the number of bacteria is high and in the colon it is even higher. In the upper intestine, the bacteria count is below 10^5 (cfu) per ml of content. This indicates comparatively light density of flora. In the mid ileum, there is a rise in the bacteria count, reaching 10^7 cfu per ml of contents. This points to an alteration in density, moving towards the thick flora present in the colon (de Vrese and Schrezenmeir, 2008).

The symbiosis in which the microflora of the intestine live in is complex and fragile. As long as their balance is maintained, the bacteria that have the potential to be harmful are contained. On the other hand, many factors in life, like inappropriate diet, antibiotic treatment, harmful bacteria, and surgery can disrupt this state of equilibrium. Other factors which can contribute to the disruption of this balance are too much travel, illness, and growing old. Whenever this state of equilibrium is upset within the intestine, the harmful bacteria find a chance to thrive and multiply. The growth of these bacteria causes digestive ailments and illnesses.

3-3-2 The gut immune system

Probiotics modulate both the GI tract's microflora and the immune system (Gorbach, 2000; Isolauri et al, 2000; Viljanen et al, 2005). The intestine functions as an extended mucosal surface, where the "interior" of the body comes into contact with the outside. It is along this important interface that alien microorganisms and antigens that take up residence or pass through the GI tract come into contact with body's vital immune system. Working against this 'colonization' of foreign elements is a fundamental activity of the intestinal flora. As soon as a foreign microorganism attaches to the intestinal surface, the epithelial layer acting as a very important obstacle against invasion stops these bacteria from merging with the eukaryotic cells present in the host (Walker, 2000; Walker, 2008).

It is essential that this obstacle structure remain healthy and functioning for the health of the individual. Malfunction and disease result when there is an alteration in the composition of the microflora of the gut or in the GI barrier. Microbes that normally live in the gastrointestinal tract strengthen this important role as well by preventing the foreign microorganisms and antigens from passing through the intestinal wall into blood circulation.

It is suggested that it reduces infections and perhaps reactions to antigens (Hooper and Gordon, 2001). It is in this intricate scheme of cooperation that the fine balance between the microflora and the gastrointestinal tract is sustained.

The immune and protective defense of the human GI tract includes the following components in the gut:

- *Immune system: It acts through a complex set of mechanisms generating certain WBC that create antibodies to take on the foreign harmful microorganisms.*
- *Mucosa and epithelium: They act as a physical blockage and sustain the intestinal permeability balanced. At the same time, they stop the harmful bacteria from attaching to the intestine's wall.*
- *Microflora: Its role is to guard the body against harmful microorganisms*

The high metabolic activity of the microbiota in the intestine is another function important to the host. Reduction in the acidity of the gut creates a harsh milieu for pathogenic bacteria. This in turn helps the good bacteria in their resistance against harmful organisms, as mentioned above

These bacteria are further able to produce antimicrobial compounds (such as peroxide, bacteriocins, and SCFA) that stop pathogens. The beneficial bacteria release SCFA, on which the immune cells also rely. Acetate, propionate and butyrate are three types of SCFAs that are produced as a result of fermentation activity by the bacteria. Butyric acid is viewed as one of the most significant substances derived from this process. The reason for this is that this acid is an ideal source of energy for epithelial cells. It is believed that butyric acid has an important responsibility in lowering the chances of getting colon cancer. Within the colonic lumen, clustering of short chain fatty acids aids in preserving a suitable pH. This is vital for effectiveness of numerous enzymes and for inhibiting metabolism of harmful or cancer causing agents in the gut (Candela et al, 2008). The role of the mucous in the intestine is to shield the epithelium, thus stopping enzymes, acid and bile from inflicting harm.

The intestinal epithelium is an added obstacle that protects it against the attack of microorganisms and foreign elements. As the location where all sorts of immunocompetent

cells function, the intestinal mucosa has a vital role in the human immune system. Nearly 80 percent of all cells which produce immunoglobulin in the body are produced by the gut-associated lymphoid tissue (GALT) (Dubois et al, 2005; Granger et al, 2006). Peyer's patches in the intestine are specialized to gather antigens from the epithelium in the GI tract. They render them to the immune system and trigger a defensive reaction. Interactions between useful bacteria or their metabolic products and the GALT in the bowel stimulates it (MacDonald and Gordon, 2005; Ouwehand et al, 2002). Research done on animals that were reared under sterile conditions has shed light on the significance of the intestinal microbiota in the growth of the gastrointestinal tract and its role in our bodies' defense mechanism against harmful microorganisms and pathogens.

A number of probiotic strains are capable to stimulate local and peripheral immunity. *Lactobacilli* are able to enhance both cellular and humoral immunity through phagocytes, macrophages, neutrophils, NK cells, and monocytes. (Vanderhoof, 2001).

L. casei has shown the capability to stimulate immune system and to increase phagocytic activity (Nagao et al, 2000; Maassen et al, 2000). In addition *S. boulardii* has demonstrated the ability to induce the immune system and stimulate activity of neutrophils, leukocytes, complement component (Guslandi et al, 2000). *L. casei* has shown ability to improve IgA response in Crohn's disease (Gupta et al, 2000).

3-4 Some potential health benefits of probiotics

There are a variety of proposed beneficial health effects of probiotics. Probiotics seem to be able to help in treatment of diarrhea, lactase intolerance, gastroenteritis, allergies in infants, hyperlipidemia, inflammatory bowel disease (IBS), irritable bowel syndrome (IBD), cancer, depressed immune function, *Helicobacter pylori* infections, and others. Some of the health benefits of probiotics will be discussed below.

Allergies/eczema - Over the last 35–40 years, the prevalence of allergic diseases has increased especially in Western countries. In babies, GI disorders are usually observed when symptoms of atopic eczema – that inhibits growth - are present. Research has mainly been done on how probiotics may affect allergies. In fact, probiotics may have positive effects on allergies through enhancing mucosal role as a barrier and boosting stimulation of the immune system (MacFarlane and Cummings, 2002). Probiotics are effective in hypersensitivity related inflammation among patients allergic to certain foods and those with atopic eczema (Majamaa and Isolauri, 1997; Murch, 2001; Pohjavuori et al, 2004; Isolauri, 2004; McFarland, 2000).

In newborn babies, the initial bacteria to colonize the sterile gastrointestinal tract may cause a permanent niche there. They will have continuing effect on immune regulation and later development of atopic disorders. Administration of *Lactobacillus rhamnosus GG* before birth has demonstrated a reduction by 50 percent in subsequent incidence of eczema in infants that are at risk (Isolauri et al, 2000). The health of infants with eczema notably improved when they were fed *Lactobacillus GG*-fortified hydrolyzed whey formula for a month. This was an indication of probiotics' ability to stimulate the gut's own barrier function that would reduce inflammation in the intestine and curing food induced allergy (MacFarlane and Cummings 2002; Kalliomaki and Isolauri 2004; Del Miraglia and De Luca 2004).

Probiotic bacteria may further be able to have a role in alleviating allergy symptoms caused by proteins in milk. The positive effect of probiotics in the above mentioned case may be due to breaking down milk proteins into smaller amino acids and peptides, thereby alleviating atopic dermatitis symptoms (Majamaa and Isolauri, 1997). Probiotic bacteria have further demonstrated the ability to upregulate anti-inflammatory cytokines, such as IL-10, in children with atopic *eczema* (Pessi et al, 2000). Other researches on animals and humans have demonstrated these bacteria's favorable effect on the immune system (Savilahti et al, 2008).

Diarrhea - A number of studies have shown that when patients suffering from acute diarrheal disorders, such as traveler's diarrhea, rotavirus infection, diarrhea related to

Crohn's disease and AIDS, and other severe bacterial infections such as *Clostridium difficile* are treated with probiotics, there is improvement in their condition (Gionchetti et al, 2000; Vanderhoof, 2001; Szajewska et al, 2001). Significantly, studies in which *saccharomyces boulardii* or *lactobacillus* species were given to patients, showed a decrease repeated episode of *Clostridium difficile*-induced diarrhea (Schultz and Sartor, 2000).

Clinical studies have shown that certain bacteria strains such as *bifidobacteria spp.*, *lactobacillus reuteri*, *lactobacillus GG*, are very effective in alleviation of diarrhea symptoms (Benchimol and Mack, 2004). Lactic acid bacteria are able to release a variety of enzymes into the GI tract that have synergistic effects on ingestion, and reducing symptoms of intestinal disorders (Gionchetti et al, 2000; Levy, 2000; Saavedra, 2000; Marteau et al, 2001).

Probiotic therapy has shown positive results in treatment of rotavirus infection and in reducing the length of acute phase of the diarrhea among children suffering from the disease (Solga, 2003; Tomas et al, 2004).

Probiotics, by their presence on the epithelial cells, may hinder activities of harmful bacteria and viruses or, by releasing bacteriocins like nisin, may prevent their growth (Del Miraglia and De Luca 2004). Also, probiotics fight the harmful bacteria over binding sites (Saulnier et al, 2009). In addition, administering probiotics can enhance the immune system.

Irritable Bowel Syndrome (IBS) - Studies have demonstrated that probiotic bacteria can be helpful in treatment of functional and inflammatory bowel disorders. Normal gut physiology is shaped by the interaction between the intestine's microbiota and the host's gastrointestinal tissues. This includes secretion, absorption, motility, and permeability (Verdu and Collins 2004). In clinical studies on IBS, a common disorder, *L. plantarum* 299v and *DSM 9843* strains were demonstrated to reduce symptoms such as bloating, abdominal pain, constipation, and flatulence (Steidler et al, 2000; MacFarlane and Cummings 2002). It has been reported, though *Sacc. boulardii* was able to reduce diarrhea in IBS, was not helpful in reducing the symptoms (Marteau et al, 2001).

***Helicobacter pylori* infections** - Unlike other microbes, a few lactobacillus strains are able to stand low acidity and stay alive and flourish under the harsh conditions in the stomach. This may provide a chance to eliminate the excess growth of *Helicobacter pylori*, and prevent conditions such as peptic ulcers and chronic gastritis. Aiba et al (Aiba et al, 1998) demonstrated that *Lactobacillus salivarius* can prevent *Helicobacter pylori* growth by making lactic acid in excessive quantity. There are contradictory results on administration of probiotics to *Helicobacter* infected patients (Franceschi et al, 2007; Ryan et al, 2008). Administration of probiotics food over an extended period of time may reduce acute GI inflammation and hence be effective in the treatment of the infection.

Alleviation of lactose intolerance - ***Alleviation of lactose intolerance*** – People with β -galactosidase (lactase) deficiency cannot tolerate milk and dairy products, except yoghurt. The fact that these individuals can tolerate fermented milk is believed to be due to the LAB present in the yoghurt that stimulates lactase activity in the small intestine (De Vrese and Schrezenmeir, 2008).

HIV and immune function – In vitro and in vivo studies on animal and human systems have indicated that probiotic bacteria, by increasing cytokines and immunoglobulin productions and by activating natural killer cell and macrophages, can improve nonspecific and specific immune responses (Perdigon and Alvarez 1992; Ouwehand et al, 2002).

Some LAB may be helpful in managing AIDS symptoms. *L. plantarum* 299v have shown to improve the immune system and individuals whose immune systems have been damaged, have benefitted from it. Feeding the bacteria to children with HIV who suffer from occasional diarrhea and malabsorption due to excess growth of bacteria, has shown promising results (Cunningham-Rundles et al, 2000; Trois et al, 2008).

Disseminated systemic “*Candida albicans*” were reduced by *bifidobacteria* and *lactobacillus spp. bifidobacteria* in the “immunodeficient euthymic mouse model” (Wagner et al, 1997). Consumption of products containing probiotics by patients whose immune systems have been compromised looks promising. However, as few tests are done on human

immune function, one should not draw conclusions on the effects of probiotic bacteria on humans, especially those with compromised immune systems (Ledoux et al, 2006).

Urogenital Infections - Some studies show the potential effects of probiotic strains of some *lactobacilli* in treatment of urogenital infections, such as bacterial vaginosis and urinary tract infections (Hoesl and Altwein, 2005; Falagas et al, 2006).

Hyperlipidemia - Another hitherto unknown benefit of probiotics is its ability to reduce serum lipid. Accumulating evidence suggests that probiotic bacteria may have a positive effect on the level of lipids in blood. It was thought that the probiotics' effect on cholesterol assimilation was "strain-dependent". Since in vitro studies demonstrated certain bacteria could take out cholesterol from culture media (Gilliland and Walker 1989; Lin and Chen, 2000; Parvez et al, 2005). Other studies have focused on the cholesterol-lowering ability of probiotics in humans (Tamime, 2002).

This may have important implications in prevention of cholesterol reabsorption back into systemic circulation. Studies ranging from in vitro to animals to humans have produced mixed results. One of the main goals of this study is to assess the effects of probiotic yoghurt on lipid profile. In the following section the effects of probiotics on serum lipids will be reviewed in depth.

3-5 Effects of probiotics on plasma lipids

The idea about the health advantages of fermented milk products in humans go back to the early 19th century. Then, Metchnikov proposed that fermenting milks by lactic acid bacteria "prevented intestinal putrefaction" and "helped maintain the forces of the body" (St-Onge, 2000). A study on Maasai tribesmen in Africa who have low serum cholesterol showed that they rarely experience coronary heart diseases, despite eating a great deal of meat. They regularly consumed 4-5 liters of fermented whole milk per day. This provided the motivation for investigating fermented milk's possible influence on blood cholesterol (Mann and Spoerry, 1974).

Later, in a study by Mann (Mann, 1977), on twenty-six volunteers, it was found that large amount of yoghurt reduced cholesterolemia, which could be due to a factor in yoghurt that prevents production of cholesterol from acetate. This factor may be either orotic acid or 3-hydroxy-3-methylglutaric acid plus thermophilus milk or methanol soluble of thermophilus milk.

3-5-1 In vitro and animal studies

Gilliland et al. showed that two strains of *Lactobacillus acidophilus* (removed from swine) were able to grow while bile was present (Gilliland et al, 1985). One of them utilized cholesterol from culture media under anaerobic conditions and considerably hindered any upward movement in serum cholesterol in pigs on high-cholesterol diet. The second bacteria did not show any effect on cholesterol in culture media or in the pigs. The researchers' conclusion was that these bacteria make it possible for cholesterol to be bound to intestine's lumen and as a result decrease its absorption. Lactose, casein, orotic acid, and hydroxymethyl glutaric acid are believed to have hypocholesterolemic effect.

Tahri et al. (Tahri et al, 1996) investigated assimilation of cholesterol by "*B. longum*, *B. infantis*, *B. breve*, *B. animalis* and *B. thermophilum*" in the presence of bile salts and observed that the removal of cholesterol from the growth medium by *bifidobacteria* strains is caused by both bacterial activity and precipitation of cholesterol.

Lin and Chen (Lin and Chen, 2000) investigated cholesterol-reducing abilities of six strains of *L. acidophilus* and found that in vivo hypocholesterolaemic ability is probably because of the assimilation of cholesterol by *L. acidophilus* cells or its attachment to the surface of *L. acidophilus* cells.

Grunewald (Grunewald, 1982) conducted an experiment, where rats were fed with fermented milk, to study the growth response and lipid profiles. Rats were divided into three groups: water (control); water plus 10% milk, and water plus 10% fermented milk. Fermented milk was prepared with probiotic strain *L. acidophilus*. Though the results were non-significant, there was a strong reduction in serum cholesterol of fermented milk-fed rats, indicating that cholesterol level in serum can be reduced by consumption of probiotics.

In another investigation, rat chow plus water, milk, or skim milk, and milk enriched with *S. thermophilus* was given to rats for 29 days (Rao et al, 1981). Plasma cholesterol concentrations were significantly higher in the group that was fed water or skim milk as compared to the thermophilus milk. The study further suggested that orotate metabolites that were created in the fermentation process in *S. thermophilus*-fermented milk could cause reduction in cholesterol concentration.

It was also seen that yoghurt decreased hypercholesterolemia that had resulted from high cholesterol in food given to rabbits (Kumar and Christopher, 1989). Their serum cholesterol elevation showed a 90% decrease in rise when *L. sporogenes* were administered to them. It was shown in in-vitro studies that *L. sporogenes* utilized cholesterol from the culture medium. This means that it may be able to use cholesterol directly from the GI tract (Kumar and Christopher, 1989).

A different study conducted on mice with high cholesterol demonstrated that *L. reuteri* was able to reduce blood lipid. Low levels of the bacteria given to the mice for a week reduced their triglyceride by 38% and their cholesterol by 40%. It also raised their HDL/LDL cholesterol ratio by 20% (Taranto et al, 1998).

Non-fat milk fermented with *L. casei shirota* was given to Syrian hamsters for 2 weeks. It caused a reduction in triglyceride level in hamsters that were given a diet with no cholesterol and also in those fed a high cholesterol diet, compared to control group (Kikuchi-Hayakawa et al, 2000).

Xiao et al examined the effects of probiotic enriched milk products on blood lipids in rats (Xiao et al, 2003). They used fermented milk contained *bifidobacterium longum* strain *BL1*. Rats were given cholesterol-enriched food. Their diet was enriched with lyophilized milk powders, as follows: 1)- acid milk, 2)- milk fermented with a culture of normal yoghurt made up of *thermophilus*, *streptococcus*, and *lactobacillus delbrueckii subsp. Bulgaricus*, and 3) *bifidobacterium* milk fermented with *B. Longum* strain *BL1*. In rats, consumption of *bifidobacterium* milk led to a meaningful reduction in their triglyceride, low density lipid, and total cholesterol, compared to the control group. The group that was fed ordinary

fermented milk, their HDL cholesterol level remained unchanged. This group showed a minor drop in its lipid levels.

Abd El-Gawad et al in a study placed rats on a high cholesterol diet for six weeks (Abd El-Gawad et al, 2005). They were fed yoghurt and soy-yoghurt containing *B. lactis Bb-12*, or *B longum Bb-46*. The groups which were given *B. lactis Bb-12* showed much lower cholesterol in their plasma and liver than did the group on cholesterol diet alone. *Bb-46* demonstrated to have more cholesterol-lowering effect than *Bb-12*. This underlines the significant differences among the various bacteria strains.

3-5-2 Human studies

Studies, on effects of fermented dairy products on human cholesterol have begun in the 1970s. Hepner et al (Hepner et al, 1979) investigated the result of consumption of yoghurt on plasma lipid level in humans. In order to uncover the effect milk products had on serum triglycerides and cholesterol, 54 subjects were studied for different durations, during which they were given dietary supplementation of non-pasteurized yoghurt, pasteurized yoghurt and 2% butterfat milk. After consumption of either non-pasteurized or pasteurized yoghurt for one week there was a significant reduction in serum cholesterol by 5 to 10 percent. However decrease in cholesterol was less significant in butter milk consuming group.

Lin et al (Lin et al, 1989) conducted two studies. One was a pilot study with no placebo, and the other was a large placebo-controlled study. In the first one, twenty-three participants were given tablets that contained (3×10^7 CFU) *L. bulgaricus* and *L. acidophilus* for 16 weeks every day, while fifteen participants were given none. Blood cholesterol was reduced significantly in the experimental group. A second study – placebo controlled, double blind, with a crossover design – was conducted. No significant change in blood cholesterol was seen.

In another study hyperlipidemic patients that were given lactobacillus *sporogenes* for 90 days, showed a 35% and 32% decrease in their LDL and total cholesterol levels, respectively (Mohan, 1990).

In a randomized, double blind, placebo controlled trial, a milk product (Gaio), fermented with two strains of *S. thermophilus* and *Enterococcus faecium*, and was examined for its effects on lowering cholesterol (Agerbaeck et al, 1995). Healthy men with normal serum cholesterol levels were told to add 200 ml/day of FM to their usual diet over a six-week period. The placebo group consumed milk acidified with an organic acid. In the fermented milk consuming group, there was a significant decrease in total cholesterol of 3% and 6% after 3 and 6 week of administering the product, respectively, and LDL cholesterol levels decreased by 10% after 6 week of consumption.

Two controlled clinical trials were conducted to study the effects of eating a single daily serving (200 ml) of fermented milk containing *Lactobacillus acidophilus* L1 taken from humans or fermented milk with *L. acidophilus* ATCC43211 from swine on lipids (Anderson and Gilliland, 1999). The first study was single-blind and lasted for 21 days. The result of first trial showed a significant (2.4%) decrease in blood cholesterol for FM containing *Lactobacillus acidophilus* L1. The second part was a placebo-controlled, double-blinded, cross-over study. In the second part of the study subjects consumed either fermented milk or FM containing *L. acidophilus* L1 over 4 weeks. In the second trial, *L. acidophilus* L1 caused a 3.2% ($P < 0.05$) decrease in blood cholesterol during the first interval. In the second interval, cholesterol remained unchanged. Collective analysis of the two L1 treatment trials revealed 2.9% reduction in blood cholesterol level (Anderson JW, Gilliland SE. 1999).

In a randomized, 2-month, double-blind clinical study, effects of fermented yoghurt on serum cholesterol of overweight individuals was examined. They consumed 450 ml/day yoghurt fermented with *Strep. thermophilus* and *E. faecium*. Results showed an 8.4% decrease in low density lipid cholesterol and an increase in fibrinogen concentrations (Agerholm-Larsen et al, 2000).

In a randomized clinical trial, 32 subjects with serum cholesterol 220-280 mg/dl were recruited. After a four-week consumption of 300 ml/day ordinary yoghurt or ordinary yoghurt starters plus *Bifidobacterium longum* strain BL1, sixteen of the participants in the probiotic group showed a decrease in their total cholesterol. Participants with relatively high total cholesterol (> 240 mg/dl) showed a particularly significant reduction in their blood lipid. Serum cholesterol levels in the rest of the probiotic group remained almost stable during the experiment (Xiao et al, 2003).

In a randomized, 56-week, double blind, placebo controlled clinical trial, the effect of enterococcus *faecium*, enriched with selenium on plasma lipids was studied (Hilvak et al, 2005). The result demonstrated that *E. faecium M-74* probiotic strain reduced cholesterol levels by 12%. As high density lipid and triglyceride concentrations showed no significant change, the decrease in serum cholesterol must be mainly attributed to a drop in low density lipid cholesterol.

In a cross-over clinical trial the effects of probiotic and conventional yoghurt on serum lipids of women with normal cholesterol concentrations were studied (Fabian and Elmadfa, 2006). Volunteers were given 100g/day of probiotic enriched yoghurt (*L. case*) or conventional yoghurt for 14 days and, without a washout period, another two weeks of 200g/day. A number of lipid levels were affected in both the conventional and the probiotic yoghurt groups, but no meaningful difference was seen between the two.

Twenty-six volunteers were recruited for a placebo-controlled, double-blind, randomized crossover study (Klein et al, 2008). After a 21-day run-in period, thirteen of them were given 300 g/day of probiotic yoghurt with *lactobacillus acidophilus 74-2* and *bifidobacterium animalis subsp. lactis DGCC 420*. The rest of the group was given placebo for five weeks. The two groups switched in the next five-week trial. Even though *L. acidophilus* and *B. lactis* were recovered in feces in significantly elevated number after supplementation, fecal SCFA and serum cholesterol levels were not changed. A significant reduction (11.6%) in serum triglyceride levels were observed, however, during the period probiotics were consumed.

In a randomized, single-blind, placebo-controlled, parallel-arm trial, fifty-five individuals with normal cholesterol levels, eighteen to thirty six years of age were selected (Greany et al, 2008). Volunteers was given either three probiotic capsules each containing 10^9 (cfu) *lactobacillus acidophilus* and *bifidobacterium Longum* and 10–15 mg fructo-oligosaccharide, or three placebo capsules per day for 60 days. The results showed no

change in plasma concentrations of triglyceride, HDL, LDL, and total cholesterol in any group.

Summary

In vitro experiments with *bifidobacteria* and *lactic acid bacteria* indicate some strains of these bacteria are able to assimilate cholesterol when bile is present. The capability of some strains to lower the level of cholesterol in humans needs to be verified in mixed substrate environments and cultures. Extensive studies done on animals and humans indicate that dairy product fermented with the right strains of LAB have cholesterol lowering activity. It is the bacterial content, according to most of these studies that produce this hypocholesterolemic ability.

Furthermore, some studies have indicated that combinations of different bacterial strains are more effective in lowering cholesterol than a single type. The differing results obtained from in vivo studies on the effect of fermented milk and yoghurt on cholesterol can be explained, at least in part, by various strains that have been used. Another important factor in successful application of probiotics is their ability to survive in the gut and colonize in the small intestine. The fact that confounding factors are also active in the environment makes reaching a decisive conclusion so much more difficult.

3-5-3 Mechanism of action

Before discussing the probiotics' mechanism of action on plasma lipids, a summary of lipoprotein synthesis and metabolism will be reviewed.

3-5-3-1 Plasma lipoprotein synthesis and metabolism

Important organs in the body that are responsible for synthesis and transport of lipoprotein are the liver and the gut. A cystic duct brings bile from the gallbladder to the gut. The liver produces the bile, but it is moved to the gallbladder and remains there to be used. Once a fatty meal arrives at the small intestine, bile salts get into action and help with emulsification of the fats. This makes their digestion and absorption in the gut possible. Fatty acids, triglycerides, and cholesterol combine in the epithelial cells of the gut where they are covered with a layer of protein. These are called chylomicrons (Kaplan & Pesse, 1996).

The lymphatic system absorbs these chylomicrons and later releases them into the blood. Chylomicrons find their way to the liver and it turns them into triglyceride and cholesterol. Bile salts do not end up in the gut with the fats. They move down all the way to the ileum. There, most of the bile salts are absorbed once again and entered into the blood. The circulation takes the bile salts back to the liver. They remain in the gallbladder, with bile, to be used for the above process again. Some of the bile salts are not absorbed in the small intestine and end up in the colon and are disposed of with feces. The liver makes up for its lost of the bile salts by synthesizing them from its cholesterol reservoir.

Cells in the liver also synthesizes cholesterol and are therefore another major source of the body's cholesterol pool, in addition to the dietary sources of cholesterol. A number of factors, such as genes and diet, stimulate the liver to produce cholesterol.

Biosynthesis of Cholesterol – Just under 50% of the body's cholesterol comes from new biosynthesis, nearly 10% in the liver, and 15% in the intestine (Kaplan & Pesse, 1989). “Cholesterol synthesis occurs in the microsomes and cytoplasm from the two-carbon acetate group of acetyl-CoA” (Dessi and Batetta, 2003). *The biosynthesis of cholesterol goes through the following stages:*

1. “Conversion of Acetyl-CoAs to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA),
2. Conversion of HMG-CoA to mevalonate,
3. Changing of mevalonate to isopentenyl pyrophosphate,
4. Changing of isopentenyl pyrophosphate to squalene,
5. Conversion of squalene to cholesterol.” (Dessi and Batetta, 2003)

Regulating Cholesterol Synthesis – In healthy adults, about 1 gram of cholesterol is synthesized and 0.3 gram is consumed per day. The body maintains a relatively constant amount of cholesterol (150-200 mg/dL). This is done mainly through controlling the level of *de novo* synthesis. Dietary intake of cholesterol in part regulates the level of cholesterol synthesis. Both of these cholesterol are then used in the formation of membranes and in the synthesis of the steroid hormones and bile acids (Croft et al, 1988). Bile acid synthesis uses most of this cholesterol.

Three separate mechanisms regulate the body's constant supply of cholesterol from cells (Kaplan & Pesse, 1989):

1. "*Regulation of HMG-CoA Reductase (HMGR)*"
2. "*Regulation of extra intracellular free cholesterol via Acyl-CoA cholesterol Acyltransferase (ACAT)*"
3. "*Regulation of cholesterol levels in plasma via HDL-mediated reverse transport and LDL receptor-mediated uptake*"

The cholesterol pool of the liver is used in two important ways. The liver utilizes part of it to produce bile salts, to be stored in the gallbladder as a part of the bile and ends up in the gut. There, the bile salts are involved in the emulsification of fats and their ingestion and absorption. The rest of the cholesterol is used for other requirements of the body. To do this, the liver combines cholesterol from its pool with triglycerides and covers it with a particular protein so that it could be dissolved in the blood. These are somewhat large molecules, known as VLDL (very low density lipoproteins). The liver then drains them into the blood.

Lipoprotein lipase (LPL) exists in abundance all over the body, especially in the walls of the arteries. This enzyme is involved in removing triglycerides from VLDL cholesterol. In the process, the VLDL shrinks in size and a relatively larger portion of it is made up of what is called intermediate density lipoproteins, or IDL.

Low Density Lipoprotein (LDL) – As the process continues, and more triglycerides are taken away, what is left is a dense molecule referred to as low density lipoprotein (LDL). This lipoprotein still maintains a large amount of cholesterol. The protein layer allows the tissues to use this cholesterol. It is the receptors on these tissues that make this interaction possible.

In the tissues such as that of the liver, and the inner layer of the arterial wall cholesterol is taken away from low density lipoproteins.

Free radicals in the body are very reactive and oxidative compounds that can oxidize low density lipoprotein cholesterol and help atherosclerotic plaque to form in the arteries. Antioxidants in the body can inhibit this process (Jialal, 1998). Vitamins such as A, C, and E, and other nutritious food we eat are important sources of antioxidants. Antioxidants are able to fight production and oxidation of free radicals. Some of these vitamins such as A, E, and C help to decrease LDL cholesterol oxidation and lower generation of these radicals.

Oxidized low density lipoproteins are not generally wanted. This is because macrophage cells can readily identify them with their receptors. When there is an inflammation due to injury, they gang up on the site. The endothelial layer in large and medium cells of the arteries is one of such sites. There, with the help of their receptors, macrophages hunt and absorb the oxidized LDL. This turns them into foam-like cells that can not function any longer and perish in the tissues. This is the early indication of atherosclerotic plaque in the arteries.

High Density Lipoprotein (HDL) – The liver also produces another type of lipoprotein, named high density lipoprotein. This is different from VLDL, which is also produced in the liver. It has little triglyceride and cholesterol, and has a particular protein covering. High density lipoprotein collects the surplus cholesterol that cholesterol metabolizing cells can not utilize. Lecithin-cholesterol acyl transferase is an enzyme that is responsible for transporting surplus cholesterol back to HDL molecules. Unused cholesterol from arteries, liver, and other tissues are absorbed by HDL cholesterol. There is evidence that even some oxidized LDL can be removed by the LCAT and HDL cholesterol (Höckerstedt et al, 2004). As HDL circulates in the body and collects the cholesterol from tissues, it becomes mature and goes back to the liver. There, it is identified by its lipoprotein covering and is lodged in the liver's cholesterol pool.

Apo A-I – Apo-A-1 is the main apolipoprotein in HDL cholesterol and performs a key function: it collects surplus cholesterol from the outer cells and transports it back to the liver. It also has antioxidant and anti-inflammatory properties (Nissen et al, 2003). Apo-

B/Apo-A ratio is an indicator of cardio vascular risk. The higher the ratio, the higher the probability of cholesterol deposits in the walls of the arteries (Walldius and Jungner, 2004).

Apo B – Apo B is found in all of the atherogenic particles; VLDL, IDL, as well as large and small dense LDL cholesterol. They all have one Apo-B molecule inside them. The number of Apo B, therefore, is an indicator of the number of the above particles. Apo B helps to capture these particles from the walls of the arteries. On the other hand, the Apo B formed in the liver helps with stabilization and transfer of cholesterol and triglycerides in plasma IDL, VLDL, and sd-LDL, and with the collecting of cholesterol in the liver and the outer tissues. Of all the Apo B particles in the blood, over ninety percent are in low density lipid cholesterol. Low to normal LDL cholesterol may indicate an increase in highly atherogenic sd-LDL particles that are readily oxidized, leading to increased formation of plaques on the arteries walls. Apo-B/Apo-A ratio is an indicator of cardio vascular risk. The higher the ratio, the higher the probability of cholesterol deposits in the walls of the arteries (Walldius and Jungner, 2004).

3-5-3-2 Probiotics' mechanism of action on lipids

It has been proposed that when probiotics settle in the gut, they ferment indigestible carbohydrate from food. Their action raises the short-chain fatty acids (SCFA) in the gut (St-Onge et al, 2000). SCFA are produced from peptide, polysaccharide, protein, and oligosaccharide, mainly by anaerobic bacteria, and are the final product of bacteria's activity in the GI tract. In terms of quantity, carbohydrates are the main source of short-chain fatty acids (ST-Onge et al, 2000). These large molecules get depolymerised by a variety of hydrolytic enzymes that are produced by bacteria and allow the organisms to ferment their sugar content. SCFA can lower the lipids in blood through blocking synthesis of hepatic cholesterol and/or through redirecting plasma cholesterol toward the liver (De Preter et al, 2006).

A hundred to 450 mmol of the SFCA is produced in the large intestine every day “with relative proportions of acetate, propionate, and butyrate being about 60:20:15 depending on the substrate” (ST-Onge et al, 2000). While Acetate seems to increase total cholesterol, propionate increases glucose in the blood and reduces hypercholesterolemia response caused

by acetate. Propionate does that by decreasing its use by the liver, for cholesterol and fatty acids synthesis.

Micelles, which play a role in the absorption of cholesterol in the intestine, are produced by bile salts, cholesterol, and phospholipids. By producing bile acids through deconjugating the bile salts in the small intestine, probiotics prevent micelle production. When cholesterol enters the enterohepatic circulation, it is dealt in the same way.

Probiotics by using hydroxy steroid dehydrogenase, and conjugated bile acid hydrolase enzymes, breakdown the bile acid and hydrolyze bile salts. By doing so bile acids' enterohepatic circulation will be disrupted (De Boever et al, 2000; Doncheva et al, 2002; Ahn et al, 2003). Hydroxy methyl glutarate CoA (HMG CoA) is another compound that helps probiotics block HMG CoA reductase activity, which is a rate limiting enzyme and is involved in endogenous production of cholesterol.

Probiotic bacteria reduce absorption of cholesterol in the intestine by binding and hence incorporating it to the cell membrane. Cholesterol can also be assimilated during growth (Noh et al, 1997). All of the above mentioned activities together help with the cholesterol lowering actions of probiotics.

3-6 Probiotics and antioxidative property

Before reviewing the studies on antioxidant property of some probiotic strains, a summary on oxidative stress will be presented.

3-6-1 Oxidative stress

Within every cell there takes place chemical reactions that involve oxidation and reduction of molecules. These result in creation of electronically charged molecules, referred to as free radicals. These charged particles need to be neutralized. The unpaired electron inside the free radicals induces them to look for and capture electrons from other molecules

(McCorde, 2000). Free radicals are normal byproducts of natural processes in the body, such as inflammation and oxygen metabolism. When cells in our body use oxygen to produce energy, for instance, free radicals are produced as a result of creation of ATP by the mitochondria. Physical exercise can contribute to the upward movement in the number of free radicals. Factors such as radiation and toxins also can contribute to the production of free radicals. Lifestyle and habits like excessive drinking and smoking, also lead to the creation of free radicals in the body. Free radicals can come together and produce other compounds that are even more harmful and destructive such as peroxynitrite (McCorde, 2000).

There are, thus, two aspects to free radicals, one is physiological and the other is pathological. On the one hand they play a role as regulatory and signaling particles and have an important role in gene transcription. On the other hand, they act as very destructive and oxidants (Fridovich, 1999). For example, nitric oxide (NO) – a nitrogen free radical – is found everywhere in our body and that participates in every single organ and cell activity. Endothelial cells produce physiologic levels of nitric oxide. That is vital for the regulation of “leukocyte adhesion, proliferation and relaxation of vascular smooth muscle cells, angiogenesis, thrombosis, platelet aggregation, vascular tone, and hemodynamic” (Ignarro et al, 1999).

Furthermore, nitric oxide that neurons produce acts as neurotransmitter, and that which stimulated macrophages produce serves as an essential mediator of the immune response (Valko et al, 2007, Fridovich, 1999). Nevertheless, Free radicals, however, that act as oxidants and disrupt the function of enzyme that have an iron-sulfur center, interact with important organic molecules such as proteins, lipids, and DNA. These biomolecules are damaged by oxidation and as a result they cannot function properly, which could cause of various diseases (McCord, 2000).

Reactive Oxygen Species (ROS) – Reactive oxygen species refers to all molecules that are extremely reactive and have oxygen in them. Free radicals belong to these species. The superoxide anion radical ($O_2^{\cdot-}$), the hydroxyl radical (OH^{\cdot}), hydrogen peroxide (H_2O_2), nitric oxide radical (NO^{\cdot}), singlet oxygen (1O_2), hypochlorite radical ($HOCl$), and lipid peroxides are different types of reactive oxygen species. They can enter into chemical reactions with

enzymes and proteins, nucleic acids, membrane lipids, and other tiny molecules, which cause cellular damage (Figure 6-3-1).

A number of pathways generate ROS. Most of the oxidants produced by cells occur as a result of: (Fridovich, 1999; Percival, 1996)

- *Phagocytes Oxidative disintegration; mechanism which bacteria, viruses and foreign proteins (antigens) are destroyed or denatured,*
- *Normal aerobic metabolism; which oxygen is used by the mitochondrial electron transport system,*
- *Metabolism of Xenobiotic; which through it lethal compounds are neutralized.*

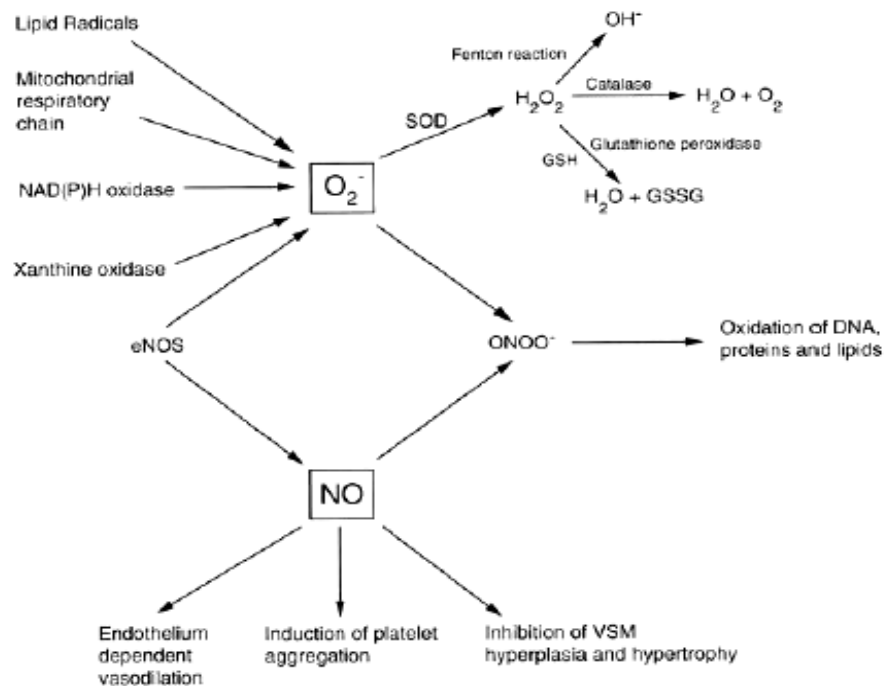


Figure 3-6-1 ROS produced in vascular tissue

O₂⁻, superoxide; NO, nitric oxide; ONOO⁻, peroxynitrite; H₂O₂, hydrogen peroxide; OH⁻, hydroxyl radical; SOD, superoxide dismutase; GSH, reduced glutathione; GSSG, oxidised glutathione; VSM, vascular smooth muscle. (Source: Hamilton et al, 2004)

Oxidative stress – Oxidative stress is seen when the creation of ROS in a system surpasses its ability to neutralize and eradicate them. This may be the result of an absence of antioxidation ability due to its disruption in production or distribution. It may also be a

result of excess ROS caused by environmental factors or high risk (McCord, 2000). The following are among the factors that may contribute to an increase in the body's oxidant load: "vigorous exercise, which fastens cellular metabolism; chronic inflammation, infection, and other illnesses; "leaky gut" pesticides, pollution, and insecticides" (Percival, 1998). If not controlled appropriately, the excess ROS can damage a cell's lipids, DNA or protein, preventing normal function.

It seems that damage done to the cells by free radicals play an important role in aging and in other related degenerative diseases, like CVD, deterioration in the immune system, brain dysfunction, cancer, and cataracts. In general these radicals are believed to be involved in initiating numerous diseases (Willcox et al, 2004; Percival, 1998)

Lipid peroxidation - One case of oxidative stress is in lipid peroxidation. The formation of lipid peroxidation products causes spread of free radical reactions Lipid peroxidation begins with hydrogen atom abstraction (Catala 2006). Hydroxyl radical, in its first reaction with PUFA produces a lipid radical (L^{\bullet}). This in turn reacts with molecular oxygen to produce a lipid peroxy radical (LOO^{\bullet}) and create a second lipid radical; a lipid hydroperoxide ($LOOH$) (Catala 2006). Lipid peroxidation can cause changes in permeability of the membrane, transportation of different ions, and also may interrupt its metabolic process. This process of peroxydation produces a whole range of compounds. This process is one of the main results of the damage done to tissues through free radicals.

Antioxidants – Formation of free radicals is naturally checked by a variety of useful compounds called antioxidants. Antioxidants in our body are the first to fight free radicals. The initial encounter leads to neutralization of free radical. Yet, another free radical is formed in this process. This begins a chain reaction. Before other free radicals are neutralized, thousands of reactions take place instantly. Antioxidants can stabilize, or deactivate free radicals before they attack cells and are vital in maintenance of systemic and cellular health. However, when there are not enough antioxidants to battle free radicals, the damage inflicted by oxidants accumulates and put the body's well being in danger.

A highly developed and complex system of antioxidant protection operates in the human body. The system has different endogenous and exogenous parts, which act interactively with

each other to combat free radicals (Valko et al, 2007). The various part of the system includes (Percival, 1998l):

- Enzymes with antioxidant properties, such as glutathione reductase, glutathione peroxidase, superoxide dismutase, which are able to catalyze free radical reactions.
- Proteins with capability to bind to metals and act as antioxidant, such as albumin, lactoferrin, ferritin, and ceruloplasmin that sequester free copper ions and iron that are able to catalyze oxidative reactions.
- Antioxidants with nutrient origin, such as ascorbic acid, carotenoids, tocopherols and tocotrienols, and other compounds like lipoic acid and glutathione.
- Other antioxidant, such as phytonutrients, which can be found in plants.

Dietary antioxidants - Among dietary antioxidants, beta carotene, and vitamins C and E have been subject of extensive studies. Vitamin C is believed to be one of the major antioxidant which is dissolvable in water and exits in extracellular fluid. It can deactivate reactive oxygen species in an early stage (in the “watery” phase), before peroxidation process (Percival, 1998). Vitamin E, on the other hand, is an important antioxidant that dissolves in fats. This vitamin is the most effective antioxidant in the cell membrane that can keep fatty acids from being peroxydized and breaks the chain. Carotenoids, especially, beta carotene shield lipid-rich tissues from peroxidation. There are other antioxidants in our diets such as phytochemicals which have been increasingly mentioned for their antioxidant activity. Flavonoids have shown the ability to act against allergens, some infections and inflammation (Balz, 2004).

Endogenous antioxidants - Dietary antioxidants are not the body’s only line of defense. A number of other mechanisms are also involved in protecting tissues from free radicals, which are endogenous. The antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase are involved in metabolizing oxidative toxic intermediates. In order to utilize their maximum catalytic potential, they need cofactors like zinc, iron, copper, selenium, and manganese.

Assessments of free radical activity - There are a number of complex methods for assessing free radical activity. There are no “gold standard” assays of free radical activity, however.

Three major approaches have been used (Jackson, 1999; Fang, 2002):

- “Measurement of endogenous antioxidant”,

- “Measurement of oxidized macromolecules products”,
- “Detection of free radicals directly”,

In order to assess endogenous antioxidant capacity, most studies have measured the plasma or cell levels of antioxidants such as carotenoids, vitamin E, folate, vitamin C, glutathione, and zinc. They have also examined the activities of enzymes with antioxidant properties, such as superoxide dismutase, glutathione reductase, catalase, and glutathione peroxidase. Because radicals and other reactive species quickly oxidize glutathione to glutathione disulfide (GSSG) and GSSG is exported from cells, intracellular ratio of glutathione (GSH) to GSSG may be taken as a reliable indicator of oxidative stress (Jackson, 1999).

Assessing peroxidation of lipids entails analyzing lipid peroxides, diene conjugates, isoprostanes, and breakdown products of lipids such as, pentane, MDA, and 4-hydroxynonenal. Of these, malondialdehyde is used most and is believed to be a true index of peroxidation of lipids. In the case of protein oxidation, the majority of researchers have made assessments based on “protein carbonyls”, “nitration of protein-bound tyrosine residues”, and the “loss of free thiol groups in proteins” (Fang, 2002). They have extensively used protein nitrotyrosine as a handy for measuring the production of reactive oxidants with nitrogen such as nitric oxide.

Certain DNA base oxidation compounds like 5-OH cytosine, 8-OH adenine, 8-OH guanine, and 8-hydroxydeoxyguanosine, have frequently been used as an indicator in order to evaluate oxidation of DNA. In addition, 8-hydroxydeoxyguanosine (urinary excretion) may give a valuable tool to determine DNA base oxidation in animals and humans (Jackson, 1999). Direct detection of free radicals has been done by “*spin trapping*” techniques and “*electron spin resonance*”.

3-6-2 In vitro studies; mechanism

Noh and Gilliland (Noh and Gilliland, 1993) have demonstrated *lactobacilli's* capacity to function as antioxidants. They have further shown that this can be measured based on how much they can shield β -phycoerythrin from oxidation by free radicals. The cell-free extracts

of the cultures showed the highest level of antioxidant capacity. This could indicate the import role of this bacteris in the GI tract in providing and releasing antioxidants.

Lin and Yen examined 19 strains of lactic acid bacteria for antioxidative activity (Lin and Yen, 1999). These bacteria included; “*Streptococcus thermophilus* 821, MC, 573, 3641, *Lactobacillus acidophilus* B, E, N1, 4356, LA-1, and *Bifidobacterium longum* B6 and 15 708, and Farr; *Lactobacillus bulgaricus* 12 278, 448, 449, Lb, 1006, and 11 842; and 19 987”. All strains showed 7-10% inhibition rate for ascorbate autoxidation. Different mechanisms were under study for the antioxidative activities were: scavenging the ROS, restriction of enzyme’s activities, chelating ability of metal ions, and intracellular cell-free extract activities of lactic acid bacteria. Among the 19 strains that were tested the utmost capability for chelating Cu^{2+} belonged to “*B. longum* 15 708” and the highest capability for chelating ion Fe^{2+} was observed in *S. thermophilus* 821. All of the 19 strains were capable to scavenge ROS. The highest scavenging activity for hydrogen peroxide was shown in *B. longum* B6, while for hydroxyl radical the highest activity was observed in *L. acidophilus* E. Although all strains showed reducing activity, *B. longum* B6 had the highest activity. None of the strains showed superoxide dismutase activity and the metal ions Cu^{2+} Zn^{2+} or Mn^{2+} , Fe^{2+} did not stimulate its activity either.

In another study by Lin and Chang (Lin and Chang, 2000) two strains of intestinal LAB; *L. acidophilus* (ATCC 4356) and *B. longum* (ATCC 15708) were studied. The antioxidative activity of intracellular cell-free extracts and intact cells of these bacteria was observed in both strains. They also inhibited linoleic acid peroxidation by 28–48%. The two strains also demonstrated that they were capable to scavenge free radical, a,a-diphenyl-b-picrylhydrazyl (DPPH). 4-nitroquinoline-*N*-oxide (4NQO) activity was inhibited by the intact cells of these two bacteria; 50% by *L. acidophilus* and 90% by *B. longum*. Intracellular cell-free extracts of none of the bacteria showed cytotoxicity inhabitation. Both strains showed between 11% and 29% protection for plasma lipid oxidation. Over all, *B. longum* demonstrated better antioxidative ability than *L. acidophilus*.

Extracellular polysaccharides (EPSs) are among major biomolecules that have antioxidant properties. Probiotic bacteria synthesize EPS. Different techniques and methods have been used to investigate the free radical scavenging ability and antioxidant activity of isolated EPS. They were compared to known antioxidants vitamin E and C, which were applied as reference standards. The results indicated a high free radical scavenging and antioxidant activities for the EPS, which was produced by *B. coagulans* RK-02 (Kodali and Sen, 2008).

3-6-3 In vivo studies

Animal studies:

Through in vitro screening, lactic acid bacteria that had antioxidative properties were chosen (Kaizu et al, 1993). Antioxidative activity of these bacteria, including *Bifidobacterium*, was assessed through an animal study using vitamin E deficient rats. Out of 570 strains, 19 showed antioxidative activity in the 1st phase of screening. In the 2nd phase, 7 of *lactobacilli* strains showed more than 70% inhibition rate of oxidation activity. *Lactobacillus* sp. SBT 2028 demonstrated the highest activity. Intracellular cell-free extracts of “*Lactobacillus casei* ssp. *rhannosus* SBT 2257” and “*Lactobacillus* sp. SBT 2028” were assessed for changes in condition of rats with vitamin E deficiency. In those rates which were fed the extract of *Lactobacillus* sp. SBT 2028, blockage in hemolysis of red blood cells was observed. This indicated that the vitamin E deficiency status was improved by the extract. An extract from *L. casei* ssp. *rhannosus* SBT 2257 showed somewhat weaker antioxidant activity than the *Lactobacillus* (Kaizu et al, 1993).

To conduct an animal study on albino rats, *Lactobacillus casei* ssp. *casei* was chosen for its high antioxidative activity through an in vitro study (Kapila et al, 2006). Examining the plasma in groups of rat that were fed fermented milk showed that their cholesterol was less by 2-11%, compared to groups that were fed skim milk. The groups that were fed lyophilized culture showed a drop in cholesterol level of 15-25%, compared with skim milk fed rats. Among rats that were given fermented milk or culture, TBARS concentrations were lower in the LDL cholesterol fraction of plasma compared to the control group fed on skim milk. The results point at the hypocholesterolemic and antioxidative potential of *Lactobacillus casei* ssp. *casei* when used as dietary adjunct.

The effect of dahi (whole yoghurt with live culture) with probiotic “*Lactobacillus casei* NCDC19” and “*Lactobacillus acidophilus* NCDC14” on worsening of “streptozotocin (STZ)-associated diabetes” in rats was examined (Yadav et al, 2008). The experiment showed that, by blocking nitric oxide and lipid peroxide formation and through maintaining antioxidant pool such as glutathione content and activities of catalase, superoxide dismutase, and glutathione peroxidase, probiotic dahi significantly suppressed STZ-induced oxidative damage, in pancreatic tissues.

Fifty-three “male Sprague-Dawley rats” were selected for a study on how probiotics affect inflammation, oxidative stress, and acinar cell (exocrine cells of the pancreas) injury in the early stage of acute pancreatitis (AP). The rats were assigned randomly to five groups: control, sham procedure, acute pancreatitis with no treatment, with probiotic treatment, or placebo (Lutgendorff et al, 2008). Oxidative stress causes injury to acinar cell and progresses the severity of AP, while prophylactic probiotics improve the condition. The study showed that AP injury and oxidative damage were improved by administering probiotics as compared to placebo. Compared to placebo, probiotics also decreased AP-induced NF- κ B and lipid peroxidation. AP-induced glutathione depletion stopped compared to placebo, and probiotic pretreatment, it even increased glutathione compared to sham rats. Biosynthesis of glutathione increased because of administering probiotics which may have lowered inflammation and injury to acinar cell.

Human studies:

Twenty-one volunteers were selected for a three-week trial in order to assess the effect of the probiotic “*Lactobacillus fermentum* ME-3” (Kullisaar et al, 2003). The volunteers were assigned into goats’ milk group or fermented goats’ milk group (150 g/d). Administration of fermented goat milk increased the protection against atherogenicity in the participants. It increased resisting period against oxidation in lipoprotein fraction, and decreased oxidized LDL level, peroxidized lipoproteins, 8-isoprostanes and glutathione redox ratio. It also improved total antioxidative activity. The quantity and ratio of lactic acid bacteria species changed in the gut microflora as a result of administration of fermented goats’ milk.

In order to examine the functional efficacy of “*Lactobacillus fermentum ME-3*”, a probiotic strain, two trials for 3 weeks were performed on healthy volunteers (Songisepp et al, 2005). The effect of two different interventions on oxidative stress markers of blood and urine, intestinal lactoflora, and fecal recovery of the probiotic bacteria were assessed. In the first open placebo controlled investigation, subjects administered either fermented goat milk by *L. fermentum ME-3* or ordinary goat milk. In the second study which was double blind randomized placebo controlled, subjects were given either placebo capsules or capsules with *L. fermentum ME-3*. Recovery of *ME-3* in feces was recognized only in the fermented milk group, by molecular methods. On the other hand, there was observed a significant increase in total antioxidative status (TAS) and total antioxidative activity (TAA) in capsules and fermented milk. Yet, the decrease of glutathione re-ox ratio values was shown only in probiotic containing fermented milk.

In order to evaluate the effect of probiotic yoghurt on oxidation parameters in plasma of human, a cross-over randomized clinical trial was carried out (Fabian and Elmadfa, 2007). Subjects were randomly allocated into two groups: conventional yoghurt and probiotic (*Lactobacillus casei*) yoghurt groups. Volunteers consumed 100 g/day of probiotic or conventional yoghurt for two weeks and 200 g/day for another two weeks. This was followed by a two-week wash-out period. The results showed a significant reduction in total antioxidant capacity values and increase in malondialdehyde and conjugated dienes (CD) values in both tested groups. While superoxide dismutase activity remained unchanged in both yoghurt groups, catalase and glutathione peroxidase activity significantly decreased in probiotic consuming group after four weeks. There were no statistically significant differences between the two yoghurt consuming groups, even though some parameters altered throughout the study.

Summary

Most lactic acid bacteria have systems to deal with oxygen radicals. Certain types of *lactobacilli* and *bifidobacteria* have been noted to produce antioxidative activity. Different antioxidative mechanisms under study in lactic acid bacteria include; scavenging the ROS, inhibiting enzyme activity, restriction of enzyme's activities, chelating ability of metal ions, and intracellular cell-free extract activities. Most strains have demonstrated very good reducing activity. Ability for chelating metal ions such as Cu^{2+} or Fe^{2+} has been shown by some LAB strains. The physiological chelators in the "intracellular cell-free extract" of LAB strains could be the reason for chelating ability of these bacteria. Other mechanism to deal with ROS is SOD and high internal concentrations of Mn^{2+} . In other species blocking the peroxy radicals is done through production of catalase. The fact that LAB can produce this minimum oxidation-reduction ability – ability required if they are to grow fully – is perhaps related to a number of the above mentioned systems.

There are very few studies on antioxidative effects of probiotics in humans. Studies reviewed in this section show promising result on the effects of some strains of bacteria on humans, but there certainly is a need for more in vivo studies on different strains of lactic acid bacteria.

4 METHODOLOGY

4-1 Study design

This study was designed as a permuted blocked randomized trial. There were three parallel groups and the study was conducted for 6 weeks. Ninety healthy women volunteers who meet the criteria to enter the study were randomly assigned into three groups, 30 in each group. The first group consumed 300 grams of probiotic yoghurt per day, the second group consumed 300 grams of conventional yoghurt, and the third group did not consume any yoghurt for 6 weeks. The study was triple blind for yoghurt consuming groups.

4-2 Sample size

Based on the equation below, the sample size needed for this study was 30 in each group, a total of 90 samples for all.

Assuming $\alpha = 0.05$, $\beta = 0.2$, $1 - \beta = 0.80$, and considering the change in mean and standard deviation of cholesterol based on a similar study ($S = 0.16$ and $\delta = 0.1$), the sample size was determined. (Ataie-Jaafari et al, 2005) The sample size was calculated based on the equation below:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 S^2}{\delta^2}$$

Based on the equation, the sample size needed for this study was calculated as 20 for each group. Considering the effect of other variables in the study, and given the differences in the results of other studies, we multiplied the sample size by 1.5. As a result, the sample size for each group was 30 and the total sample size was 90. This would increase the power of the study. Two subjects had to be excluded from the study after the first week because of taking antibiotics, one from the probiotic group and one from the control group. The demographic and blood lipid parameters of dropouts were not different than those subjects who completed the study.

4-3 Sampling Criteria

Volunteers were recruited from the students and personnel of Tehran University of Medical Sciences through advertising at the university. The inclusion and exclusion criteria to enter the study were as follow:

Inclusion Criteria:

- Triglyceride less than 200 mg/dl
- Cholesterol less than 240 mg/dl
- Body Mass Index (BMI) up to 30
- Females between 19-49 years old (pre-menopausal)

Exclusion Criteria:

- Having hypercholesterolemia, diabetes mellitus, thyroid disorders, or immunodeficiency diseases
- Having the history of a myocardial infarction, angioplasty or stroke
- Using anticoagulants, immunosuppressant, corticosteroids, thyroid replacement and hypercholesterolemia medication
- Having lactose intolerant
- Consumption of probiotic yoghurt or any other probiotic containing products in the last month
- Pregnancy or having plans to become pregnant during the time course of the study or lactating
- Smoking
- Having kidney or liver disease, inflammatory intestinal disease
- Elite athletes
- Use of any antimicrobial agent within the last month before the study, or use of any regular concomitant medication including antioxidant supplement, vitamins, and anti-inflammatory non-steroidal drugs were excluded
- Having special diet (vegetarian, vegan, etc)

Volunteers were checked to see if their complete blood counts (CBC) were normal and if their cholesterol and triglyceride level and other criteria met the criteria to enter the study. A one-week pre-adjustment period was designated, during which all subjects had to refrain

from taking yoghurt or any other fermented food. Written informed consent was taken from each volunteer who took part in this study. Subjects were randomly assigned into three groups, each group consisting of 30 individuals. The first and second groups consumed 300 grams, per day, of probiotic and conventional yoghurt, respectively. The third group, as the control group, did not consume any yoghurt for the duration of the study (45 days). Required information was given to conventional and probiotic yoghurt consuming groups on proper storage and use of the products.

The volunteers were told not to alter their daily physical activity or diet, and not to use soya milk and not to add high fiber items into fermented milk, and not to consume any other yoghurt other than the one provided. They were also asked to refrain from consuming any other probiotic products. Weekly follow up on use of study products, amount of exercise and use of any medication was performed. Necessary arrangements were made so that every week the subjects would receive a week's supply of their probiotic or conventional yoghurts directly from the factory. The Ethics Committee at TUMS approved the study's protocol.

4-4 Probiotic yoghurt

Both conventional and probiotic yoghurts were produced by the Research and Development (R&D) Division of Iran Dairy Industry Corporation, (IDIC-Pegah) Tehran. IDIC is one of the major dairy manufacturers in Iran. Probiotic and conventional yoghurts supplied, both, contained *L. bulgaricus* and *S. thermophilus*. The probiotic yoghurt was also enriched with adding cultures of *B. lactis* Bb12 and *L.acidophilus* La5 (by Christian Hansen). Direct vat set cultures were used. Microbiological analysis of the probiotic yoghurt showed that it contained 3.9×10^7 (cfu) of both *B. lactis* Bb12 and *L.acidophilus* La5. The analysis on conventional yoghurt confirmed the presence of 10^6 - 10^7 cfu of *S. thermophilus* and *L. bulgaricus*. The yoghurts' acidity was 4.3. The fat content in both yoghurt types was 2.5%.

Those in conventional or probiotic yoghurt group were provided to consume 300 gram per day. The yoghurts were presented in identical plastic cups containing 150 gram yoghurt. Each container had a different color top that indicated the intervention group. It was not known to the subjects, researcher, and analyzer which container contained probiotic or

conventional yoghurt. A third party had the enclosed intervention codes, which were revealed after the analyses were done.

4-4-1 Some characteristics of LA5 and BB12

LA-5 is facultative anaerobic, so it is able to function in the presence or absent of oxygen. It is able to grow in very acidic environments. *BB-12* is only function anaerobically. Lactic acid is not the only acid it produces. Succinic and acetic acids are also products of *BB-12*. Both of them give a feeling of desirable fresh taste.

The reason for selection of these probiotic strains (by Hansen) was because the following criteria were met:

a) Technological properties

- Single strains can be produced as highly concentrated DVS culture for the direct inoculation of milk
- Good technological characteristics in production of acidified and unacidified probiotic dairy products
- Both strains show fermentation activity in milk (i.e. among other things acidification and lactose utilization).
- The strains show a high survival ability in the unacidified and acidified product during cool storage

b) Resistance during the gastrointestinal passage; (Wang et al, 2004; Ouwehand et al, 2001)

- *LA-5* has shown a survival ability of “100% at a pH of 3.0 and 4.0 over 2 hours. At a pH of 2.0 the survival ability was reduced by 2 log values”,
- *BB-12* has shown a survival rate of “100% at a pH of 2.0. After 2 hours only a slight drop was detected”,
- In vitro studies both strains have shown a high survival rate during the gastrointestinal passage, when administrated through cultured milk products (Klaver and Van Der Meer, 1993; Noh and Gilliland, 1993).

c) Activity and adhesion in the intestine

By means of adhesion to the inner surfaces of the intestinal tract, probiotic bacteria can inhibit the colonization of other, partly pathogenic microorganisms. Furthermore, adhesion

extends the period for interactions between probiotic bacteria, intestinal flora and the intestinal tract's immune system. Good adhesion ability to the cells of the colon epithelium or to the mucosal layer which lies thereon is thus an important selection criterion for probiotic bacteria. *LA-5* and *BB-12* show good adhesion ability to human mucous (Kirjavainen et al, 1998; Ouwehand et al, 2000).

In addition lactic and acetic acid, hydrogen peroxide (H₂O₂) and bacteriocins belong to these substances. These are proteins which inhibit only a small number of mostly closely related bacterial strains without damaging the intestinal flora as a whole. Apart from small amounts of H₂O₂, *LA-5* also produces acidocin CH5, a broad spectrum bacteriocins (Chumchalova et al, 1998) which also inhibits certain yeast.

4-5 Data collection

The subjects were asked to be present the Department of Nutrition and Biochemistry of School of Public Health at Tehran University of Medical Sciences, at three intervals; at the beginning of the study, at the end of the third week, and at the end of the sixth week. Information on anthropometric measurements, food consumption (through 3-day dietary record), and fasting blood samples were collected at three intervals. Compliance with the yoghurt intake guidelines at home was monitored once a week through phone interview.

4-5-1 Anthropometric measurements

At the beginning, at the end of the third week, and at the end of the sixth week (end of the intervention) anthropometric measurements of the subjects were taken. Body weight and height were measured with Seca scale and non-stretchable tape. At each interval, body weights were measured with 0.1 kg accuracy without shoes and with minimum clothing. Heights were measured, with 0.1 cm accuracy. BMI was determined by dividing body weight by height squared (kg/m²).

4-5-2 Dietary records

Prior to the intervention, those who enrolled in the study were taught by a dietician how to record the amounts of foods that were eaten. A written instruction was also given to them. At the beginning of the study, at the end of the third, and at the end of the sixth weeks,

three-day dietary records were taken from each volunteer who was directed to write down the types and amounts of foods eaten. The amount of food eaten by each subjects was estimated from household measures and from photos of serving sizes (Ghafarpour et al, 1999). Subjects were instructed to maintain their usual dietary habits and their exercise patterns throughout the experiment. Weekly follow ups were performed by phone. A nutritionist checked the participants' dietary records in person upon their periodically visits to the Nutrition Department at TUMS for anthropometric measurements and blood sampling at the beginning of the study, at the end of third and sixth weeks of study. The dietary records were analyzed for individual nutrients (total energy, total fat, saturated fatty acid, polyunsaturated fatty acid, monounsaturated fatty acids, dietary fiber, vitamin A, C, and E) using the computer based program, Food Processor II.

4-5-3 Biochemical measurements

Blood samples were drawn from the subjects in the morning after 14 hours of fasting at the three intervals; at the beginning of the study, at the end of the third week, and at the end of the sixth week. Blood was taken from the antecubital vein in the arm. To prepare plasma, the blood (6ml) was collected into tube with anticoagulant agent; the rest (4ml) was collected into tube with no anticoagulant agent for preparing the serum. For the plasma, blood samples were centrifuged for 10 minutes at 3000 U/min. Plasma separated and red blood cells were acid washed 3 times with physiologic serum (4°C) and stored at -80°C for analysis.

For serum, collected blood samples were left for 40 minutes in 30°C to allow them to clot. Then using a glass pasteur, the clot was loosened from the sides of the tube. They were then centrifuged at 3000 U/min for 10 minutes to separate the serum from the clotted cells and the resulting serum was stored (in 5ml micro-tubes) at -80°C for analysis.

The blood was analyzed for:

Dependant variables	Methods
Total cholesterol	<i>Enzymatic</i>
HDL cholesterol	<i>Enzymatic</i>
LDL cholesterol	<i>Enzymatic</i>
Triglyceride	<i>Enzymatic</i>

Apolipoprotein A	<i>Immunoturbidometry</i>
Apolipoprotein B	<i>Immunoturbidometry</i>
Total antioxidant capacity	<i>Spectrophotometer</i>
Malondialdehyde	<i>Spectrophotometer</i>
Oxidized LDL	<i>Eliza</i>

Serum total cholesterol and triglyceride concentrations were measured with Parsazmun's kits (DiaSys, Germany) using an enzymatic method CHOD-PAP (cholesterol oxidase phenol 4-aminoantipyrine peroxidase) and GPO-PAP (glycerol phosphate oxidase phenol 4-aminoantipyrine peroxidase) (Artiss and Zak, 1997; Cole et al, 1997). Low-density and high-density lipoprotein cholesterol were also measured with the Parsazmun's kits (DiaSys, Germany) by an enzymatic method (Rifai et al, 1999). Apolipoprotein A-1 and Apolipoprotein B were analyzed by Immunoturbidometry method (Bhatnagar and Durrington, 1997). Parsazmun's kits (DiaSys, Germany) were used for Apo A and Apo B measurements.

Plasma MDA was measured as an indicator of lipid peroxidation based on the thiobarbituric acid (TBA) spectrophotometric method that was introduced by Satoh (Satoh, 1978). Total antioxidant capacity was measured according to Rice-Evans and Miller (Rice-Evans and Miller, 1994). The assay is dependent on the hindrance of the absorbance of radical cations of 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) (ABTS) by antioxidants as a result of incubation of ABTS with a peroxidase and H_2O_2 . The Mercodia Oxidized LDL Eliza kit (Sweden) was used for quantitative measurement of oxidized low density lipoprotein.

4-6 Statistical analysis

Analysis of variance (ANOVA) was used in the evaluation of the data. The ANOVA was used to indicate any difference among the three groups or among the three intervals within a group. The normality of the distribution of variables was tested using the Kolmogorov-Smirnov test. When the distribution was not normal, the Naperian Logarithm transformation of values was performed to make data fit to the normal distribution and then the ANOVA was applied.

Nonparametric test of Kruskal-Wallis was used for those distributions which were not normal. In that case, the data are expressed in medians and inter-quartile ranges (IQR) of the original variable; otherwise the data are expressed in means and standard deviations. Multiple comparisons were done with the Bonferroni post-hoc test. Linear relationships between serum values were examined using Pearson's correlation coefficient. Statistical Package for Social Sciences (SPSS/PC version 11.5) was used for the statistical tests. Food Processor (FPII, version 2) was used for calculating the dietary record (FPII, version 2). The differences were considered statistically significant at $P < 0.05$.

5 RESULTS

To compare the mean of variables among the three groups and within each group throughout the study, analysis of variance (ANOVA) was applied. The normality of the variables distribution was tested using the Kolmogorov-Smirnov test. Whenever the distribution was not normal by conducting Kolmogorov-Smirnov test, the Naperian Logarithm transformation of values was performed to make data fit the normal distribution. For distributions that were not normal the nonparametric test of Kruskal-Wallis was used to compare groups and the data were expressed in medians and inter-quartile ranges (IQR) of the original variable. Otherwise, the data were expressed as means and standard deviations. Multiple comparisons were conducted by the Bonferroni post-hoc test. Linear relationships between serum values were examined using Pearson's correlation coefficient. A P-value less than 0.05 was considered to be statistically significant.

In this chapter results will be presented in the following sections:

- 5-1 General characteristic of subjects
- 5-2 Dietary intakes of subjects
- 5-3 Blood lipid parameters
- 5-4 Blood oxidant and antioxidant parameters

5-1 General characteristics of subjects

The means and standard deviations (SD) of age, body weight, height and BMI of the subjects at the baseline are presented in Table 5-1-1. Body mass index is obtains by dividing weight in kilograms by height in meters squared. The normal range for BMI is between 20 and 25. A value above 25 and 35 are overweight and obese, respectively. In this study, the mean value of BMI was within the normal range for all the three groups at baseline. At the beginning of the study, there were no statistically significant differences in age, weight, height and BMI among the three groups - the groups consuming conventional yoghurt, probiotic yoghurt, and the control group.

Table 5-1-1. Means and standard deviations of characteristics of the subjects at baseline

Variables \ Groups	Conventional yoghurt (n=30)	Probiotic yoghurt (n=29)	Control (n=29)	P- value
Age (year)	32.0 ± 6.8	35.2 ± 6.4	34.7 ± 7.5	0.173
Body Weight (kg)	58.47 ± 6.78	60.68 ± 7.01	59.33 ± 7.26	0.478
Height (cm)	159.40 ± 5.19	158.86 ± 6.04	158.11 ± 6.37	0.698
BMI (kg/m ²)	23.02 ± 2.44	24.03 ± 2.42	23.76 ± 2.99	0.317

Means and SDs of body weight and BMI of the subjects, at the three intervals of the study; baseline (T1), end of third week (T2), and end of the sixth week (T3) were compared within each group (Table 5-1-2). Mean values for body weight and BMI of the participants were constant throughout the study for all the three groups. The ANOVA did show significant changes in body weight and BMI of the subjects at the three intervals of the study, within a group.

Table 5-1-2. Means and standard deviations of weight and BMI of the subjects within each group during the study

Variables \ Groups \ Intervals		Baseline (T1)	3 rd weeks (T2)	6 th weeks (T3)	P- value
Body Weight (kg)	Conventional yoghurt	58.47 ± 6.78	58.45 ± 7.01	58.91 ± 7.15	0.958
	Probiotic yoghurt	60.68 ± 7.01	60.76 ± 7.14	60.88 ± 7.39	0.994
	Control	59.33 ± 7.26	59.30 ± 7.20	59.33 ± 7.30	1.000
BMI (kg/m ²)	Conventional yoghurt	23.02 ± 2.44	23.01 ± 2.58	23.19 ± 2.55	0.954
	Probiotic yoghurt	24.03 ± 2.42	24.13 ± 2.52	24.04 ± 2.58	0.985
	Control	23.76 ± 2.99	23.74 ± 2.88	23.77 ± 2.85	0.999

5-2 Dietary intakes of subjects

In order to find the differences among the three groups or changes within a group, data from three-day dietary record for intakes of total energy, total fat, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acids (PUFA), cholesterol, beta carotene, dietary fiber, vitamin C, and E were studied.

The means and standard deviations of total fat, SFA, MUFA, PUFA, dietary fiber, vitamin C, and E are presented in table 5-2-1. The ANOVA did not show any difference among the three groups at baseline for intake of total fat, saturated fatty acid, MUFA, PUFA, dietary fiber, vitamin C, and E (Table 5-2-1).

USDA Dietary Guidelines of 2005 recommends that “total fat intake should count between 20 to 35 percent of energy for adults” and “no more than 10 percent of energy should be from saturated fats”. (A dietary guideline for Iranian population is under development.) Peoples’ diets vary in different parts of the world. Among the Mediterranean, fat intake could provide forty percent of the calorie. But, as saturated fat in the diet raises blood cholesterol, such diet must be low in saturated fat and have a high MUFA and fiber content. Foods from animals and some plants are high in saturated fat. One of the main causes of high blood cholesterol is dietary saturated fat. In the present study, the mean value of percent of fat from energy was between 34 to 35 percent in all groups, at the baseline (Table 5-2-1).

Polyunsaturated and monounsaturated fats appear to have a positive effect on plasma lipid profile. The National Heart, Lung, and Blood Institute recommend that up to 20% of total daily energy should be from MUFA and up to 10% from PUFA. Double bonds in polyunsaturated fatty acids can help create free radicals. These free radicals can then react with oxygen and produce lipid peroxide compounds with unstable oxygen bonds similar to those in hydrogen peroxide. Polyunsaturated fatty acids may have a protective effect against atherosclerosis but moderation in consumption is recommended.

Monounsaturated fats decrease LDL cholesterol level and other blood lipids while raising the high density lipoprotein cholesterol level. Monounsaturated fatty acids have these favorable effects on blood lipids even in diets containing as much as 35% fat, as long as one third or more of the fat is from MUFA. In the present study, the mean values of SFA,

MUFA, and PUFA from total energy were between 10 to 11 percent in the three groups (Table 5-2-1).

Fibers accelerate the movement of food in the colon and slow down simple carbohydrates' absorption. Thus, they can reduce constipation and help regulate blood sugar and insulin sensitivity. Soluble fibers bind to bile acids and reduce blood cholesterol. Soluble fiber can also lower the LDL cholesterol. Daily Reference Intake (DRI) of fiber for women is 25 grams per day. The mean value for dietary fiber was less than 25 for all the three groups but there were not statistically significant differences among the three groups at the baseline (Table 5-2-1).

Carnitine is a tiny molecule that is vital for fat to reach mitochondria to be converted into energy. Synthesis of carnitine, in turn, requires vitamin C. This vitamin is also involved in cholesterol's metabolism to bile acids and can increase the risk of gallstone (Higdon, 2003). Vitamin C is also a powerful antioxidant. The recommended amount by RDA for non-smoker adult women is 75 mg/day. In this study, in the probiotic yoghurt group the mean value for vitamin C was 72 mg but in the two other groups the amount was higher than 75 mg. There but there were not statistically significant differences among the three groups at the baseline (Table 5-2-1).

Vitamin E is a fat-soluble, antioxidant vitamin. It also plays a role in regulation of gene expression, immune function, cell signaling, and other processes related to metabolism (Higdon, 2003). There are eight forms of vitamin E in nature. They have different levels of biological activity. Only alpha tocopherol can be utilized by the human. Recommended dietary allowance for vitamin E (Alpha-Tocopherol) for adult women is 15 mg (22.41 IU). In the present study, the mean value for vitamin E was 14.48 mg for the probiotic yoghurt group, and 15 mg for the other two groups. There were not statistically significant differences among the three groups in vitamin E intakes at the beginning of the study (Table 5-2-1).

Table 5-2-1. Means and standard deviations of nutrient intakes according to 3-day dietary record at baseline for each group

Groups Variables		Conventional yoghurt	Probiotic yoghurt	Control	P- value
Total fat	(g)	64.72 ± 14.63	64.57 ± 13.74	68.54±23.46	0.629
	(% of energy)	34.02±4.96	35.74±5.43	35.72±5.58	0.369
SFA	(g)	21.19 ± 7.43	20.00 ± 5.85	22.35 ± 9.78	0.525
	(% of energy)	10.96±2.37	10.94±2.67	11.52±2.94	0.613
MUFA	(g)	19.31 ± 4.72	19.87 ± 4.77	20.42 ± 6.56	0.736
	(% of energy)	10.12±1.57	11.03±2.35	10.70±1.69	0.183
PUFA	(g)	21.96 ± 8.47	20.60 ± 6.18	21.29 ± 9.11	0.809
	(% of energy)	11.51±3.72	11.45±3.15	11.12±3.56	0.898
Dietary Fiber	(g)	16.52 ± 5.40	16.68 ± 6.66	16.85 ± 7.12	0.981
Vitamin C	(mg)	83.63 ± 54.08	72.46 ± 50.66	81.96 ± 54.81	0.689
Vitamin E (α -tocopherol)	(mg)	15.24 ± 6.49	14.48 ± 6.74	15.92 ± 9.64	0.778

For intakes of total energy, cholesterol and beta-carotene distributions were not normal. Therefore, Kruskal-Wallis test was carried out to compare the baseline values of these parameters among the three groups (Table 5-2-2). The recommended daily energy intake is 1940 calories a day for women. In the present study the median for total energy intake of all the three groups was below 1940, but there were no statistically significant difference among them.

Vitamin A is vital for the functioning of the immune system. This group of compounds is also involved in cell division, bone growth, reproduction, and vision (Higdon, 2003). Beta-carotene and other carotenoids can be converted by the body into retinol (preformed vitamin A). Beta carotene is one of the most important natural forms of antioxidants and has immune-system-enhancing activity. There is no RDA for beta carotene. There were no statistically significant differences among the three groups.

According to USDA Dietary Guidelines of 2005, recommended cholesterol intake is less than 300 mg. The median value for cholesterol intake in the three groups was less than 300 mg. There were no statistically significant differences between medians and IQRs of these parameters among the three groups at baseline.

Table 5-2-2. Medians and IQRs of energy, cholesterol and beta-carotene according to 3-day dietary record at baseline for the three groups

day dietary record at baseline for the three groups				
Groups	Conventional yoghurt	Probiotic yoghurt	Control	P- value
Variables	Median (IQR)			
Total energy (kcal)	1646.50 (579.75)	1686.00 (567.50)	1657.00 (555.0)	0.762
Cholesterol (mg)	162.50 (139.75)	146.00 (138.00)	173.00 (173.50)	0.791
β -carotene (mg)	1.75 (3.80)	1.49 (2.77)	2.00 (2.71)	0.437

The intakes of total fat, saturated, monounsaturated, polyunsaturated fatty acids, dietary fiber, vitamin C, and E within each group at the three intervals are presented in Table 5-2-3. The ANOVA did not show any significant difference during the study, in the three intervals, for intake of these parameters.

Table 5-2-3. Means and standard deviations of nutrient intakes according to 3-day dietary record throughout the study for each group

Intervals		Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value
Variables	Groups				
Total fat (g)	Conventional yoghurt	64.72 ± 14.63	71.19 ± 21.40	66.67±16.60	0.355
	Probiotic yoghurt	64.57 ± 13.74	62.47±13.96	64.17±24.27	0.895
	Control	68.54 ± 23.46	65.59±25.73	64.75±17.11	0.796

Total fat (% of energy)	Conventional yoghurt	34.03±4.96	34.67±6.86	33.90±5.32	0.859
	Probiotic yoghurt	35.74±5.43	33.55±7.09	33.81±5.86	0.342
	Control	35.72±5.58	34.98±5.61	35.11±6.31	0.878
SFA (g)	Conventional yoghurt	21.19 ± 7.43	23.05 ± 8.29	22.56 ± 7.46	0.630
	Probiotic yoghurt	20.00 ± 5.85	22.49 ±6.16	21.81±7.80	0.341
	Control	22.35 ± 9.78	19.84±7.54	20.49±6.86	0.482
SFA (% of energy)	Conventional yoghurt	10.96±2.37	11.15±2.70	11.33±2.22	0.841
	Probiotic yoghurt	10.94±2.27	11.89±2.60	11.52±2.02	0.292
	Control	11.52±2.94	10.61±1.58	10.86±1.95	0.283
MUFA (g)	Conventional yoghurt	19.31 ± 4.72	21.44± 6.86	21.10 ± 6.22	0.340
	Probiotic yoghurt	19.87 ± 4.77	18.98±4.54	19.36±7.44	0.840
	Control	20.42±6.56	19.61±8.02	19.87±5.71	0.900
MUFA (% of energy)	Conventional yoghurt	10.12±1.57	10.46±2.52	10.66±1.83	0.582
	Probiotic yoghurt	11.03±2.35	10.15±2.04	10.19±1.86	0.199
	Control	10.70±1.69	10.46±2.19	10.78±2.41	0.836
PUFA (g)	Conventional yoghurt	21.96 ± 8.47	22.53 ± 8.45	20.59 ± 9.08	0.674
	Probiotic yoghurt	20.60 ± 6.18	17.47±6.62	18.86±10.60	0.337
	Control	21.29 ± 9.11	21.93±11.02	20.07±7.63	0.744
PUFA (% of energy)	Conventional yoghurt	11.51±3.72	10.96±3.21	10.41±3.64	0.487
	Probiotic yoghurt	11.45±3.15	9.49±3.91	9.87±3.58	0.091
	Control	11.12±3.56	11.64±3.80	11.11±4.18	0.831

Dietary fiber (g)	Conventional yoghurt	16.52 ± 5.40	17.93 ± 6.46	18.29 ± 8.75	0.590
	Probiotic yoghurt	16.68 ± 6.66	16.55 ± 6.98	14.79 ± 5.40	0.458
	Control	16.85 ± 7.12	17.15 ± 7.20	17.96 ± 8.41	0.848
Vitamin C (mg)	Conventional yoghurt	83.63 ± 54.08	78.08±46.00	77.10±47.64	0.859
	Probiotic yoghurt	72.46 ± 50.66	73.32 ± 48.98	81.66 ± 44.57	0.725
	Control	81.96 ± 54.81	83.56 ± 55.84	68.49 ± 52.82	0.515
Vitamin E (mg α-tocopherol))	Conventional yoghurt	15.24 ± 6.49	16.40 ± 8.02	15.84 ± 9.75	0.861
	Probiotic yoghurt	14.48 ± 6.74	13.45 ± 7.00	13.44 ± 8.82	0.836
	Control	15.92 ± 9.64	16.46 ± 9.09	16.17 ± 6.56	0.971

In the present study, the mean value for percentage of fat from energy was between 33 and 35 in all groups, at the three intervals. The mean values for SFA, MUFA, and PUFA from total energy were between 9 to 11 percent in the three groups (Table 5-2-3).

In western countries diets the ratio between polyunsaturated and saturated fatty acids is less than one. It is believed that increasing it to near one would lower the risk of CVD. The P/S ratios in all the three groups, during the three intervals of the study are as follow:

	Baseline	3 rd weeks	6 th weeks
Conventional yoghurt	1.03	0.95	0.91
Probiotic yoghurt	1.03	0.78	0.86
Control	0.95	1.10	0.98

The lowest ratio belongs to the probiotic group at the second interval of the study (0.78), which is, higher than the ratio in diets in Western countries.

Since the distributions were not normal for total energy, cholesterol and beta-carotene, Kruskal Wallis test was used for those parameters to examine any significant change at the three intervals within each group (Table 5-2-4). The results show no statistically significant change in those parameters throughout the study within a group. So, there was no statistically significant difference at the three intervals of the study for any of the dietary parameters.

Table 5-2-4 Medians and IQRs of energy, cholesterol and beta-carotene intake according to 3-day dietary record throughout the study for the three groups

Intervals Variables Groups		Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value
Total energy (kcal)	Conventional yoghurt	1646.5 (579.7)	1788.5 (569.7)	1731.5 (785.7)	0.392
	Probiotic yoghurt	1686.0 (567.5)	1636.0 (383.5)	1723.0 (633.5)	0.926
	Control	1657.0 (555.0)	1645.0 (743.5)	1622.0 (743.5)	0.873
Cholesterol (mg)	Conventional yoghurt	162.5 (139.7)	206.5 (161.7)	195.0 (222.0)	0.608
	Probiotic yoghurt	146.0 (138.0)	199.0 (134.5)	158.0 (170.7)	0.380
	Control	173.0 (173.5)	169.0 (106.0)	142.0 (136.5)	0.518
Beta carotene (mg)	Conventional yoghurt	1.8 (3.8)	2.2 (3.2)	1.7(2.5)	0.612
	Probiotic yoghurt	1.5(2.8)	1.9 (1.8)	1.4 (2.7)	0.669
	Control	1.9(2.7)	2.7(2.2)	1.6 (2.2)	0.071

So far, two comparisons on dietary data have been presented; a) comparing baseline values between conventional yoghurt, probiotic yoghurt and control groups, and b) comparing values at the three intervals within a group. Furthermore, to detect any possible changes during the course of the investigation (6 week period) among the three groups, the differences between baseline means and sixth week means for each group were calculated and then the differences were compared among the groups. Results of comparing differences

between baseline (T1) means and sixth week (T3) means for dietary intake parameters are presented in Table 5-2-5. The ANOVA did not show any significant difference among the three groups for mean differences.

Comparing baseline (T1) and sixth week (T3) means showed that there was a decrease in the amount (g) of total fat intake of the probiotic yoghurt group and the control group and an increase in that of the conventional yoghurt group (Table 5-2-5). Comparing the percentages, however, showed decreases in three groups. There were increases in the amount and percentages of SFA in both yoghurt groups and decreases in the control group. The amount of MUFA decreased in the probiotic yoghurt group and the control group. There were increases in the percentage MUFA intakes in both conventional yoghurt and the control groups, while a decrease was observed in the probiotic yoghurt group. For PUFA, there were decreases in the three groups. Dietary fiber increased in the conventional and the control groups, but decreased in the probiotic yoghurt group. Vitamin C decreased in the conventional yoghurt group and the control group while it increased in the probiotic group. For vitamin E, there were increases in the mean values in the conventional yoghurt and the control groups and a decrease in the probiotic yoghurt group. None of the above mentioned changes were statistically significant among the three groups (Table 5-2-5).

Table 5-2-5 Differences between baseline (T1) mean and 6th week (T3) mean of nutrient intakes for the three groups

Variables \ Groups	Conventional yoghurt (T1-T3) Mean \pm SD	Probiotic yoghurt (T1-T3) Mean \pm SD	Control (T1-T3) Mean \pm SD	P- value
Total fat (g)	1.95 \pm 22.96	-0.40 \pm 26.15	-3.79 \pm 27.11	0.686
Fat (% of energy)	-0.12 \pm 6.22	-1.93 \pm 7.30	-0.60 \pm 7.69	0.603
SFA(g)	1.37 \pm 7.45	1.81 \pm 7.38	-1.86 \pm 10.08	0.192
SFA (% of energy)	0.37 \pm 2.45	0.58 \pm 1.57	-0.65 \pm 3.54	0.167
MUFA (g)	1.79 \pm 6.70	-0.51 \pm 8.30	-0.54 \pm 7.19	0.386
MUFA (% of energy)	0.54 \pm 2.09	-0.84 \pm 2.50	0.08 \pm 2.56	0.085
PUFA (g)	-1.37 \pm 10.67	-1.74 \pm 13.39	-1.22 \pm 12.22	0.986

PUFA (% of energy)	-1.10±3.69	-1.58±5.31	-0.01±4.94	0.428
Dietary fiber (g)	1.76 ± 8.70	-1.88 ± 6.31	1.11 ± 9.11	0.194
Vitamin C (mg)	-6.53 ± 54.11	9.20 ± 62.59	-13.47 ± 52.82	0.300
Vitamin E (mg)	0.60 ± 9.51	-1.03 ± 10.51	0.25 ± 10.31	0.809

Kruskal-Wallis test showed no statistically significant difference among the three groups for median differences (T1 minus T3) for energy, cholesterol and beta-carotene (Table 5-2-6). There were increases in median intakes of total energy and cholesterol in both yoghurt groups and decreases in the control group. Median intake of beta carotene decreased in all three groups.

Table 5-2-6 Differences between baseline (T1) and 6th week (T3) median intakes of energy, cholesterol and beta-carotene according to 3-day dietary for the three groups

Variables	Groups	Conventional yoghurt (T1-T3)	Probiotic yoghurt (T1-T3)	Control (T1-T3)	P- value
	Median (IQR)				
Total energy (kcal)		55.00 (784.25)	44.00 (435.00)	-62.00 (584.00)	0.721
Cholesterol (mg)		29.25 (193.85)	5.70 (149.60)	-26.00 (183.55)	0.228
Beta carotene (mg)		-0.21(2.69)	-0.29(2.87)	-0.01(2.52)	0.759

In summary, there were no statistically significant differences among the three groups and the three intervals of the study for intakes of any nutrients and energy. The mean differences between baseline and six week also did not show any statistically significant differences among the three groups for nutrient and energy intakes.

5-3 Blood lipid parameters

Blood lipid parameters of subjects including; total cholesterol, triglyceride, HDL and LDL cholesterol, total/HDL cholesterol ratio, Apo A and Apo B are presented in this section. Blood lipid parameters of the subjects at baseline are shown in Table 5-3-1. The ANOVA

did not show any statistically significant difference among the three groups for blood lipid parameters at baseline.

Table 5-3-1. Means and standard deviations of blood lipid parameters of the subjects at baseline

Variables \ Groups	Conventional yoghurt	Probiotic yoghurt	Control	P- value
Total Cholesterol (mg/dl)	173.90 ± 25.62	185.66 ± 23.86	184.38 ± 27.85	0.164
Triglyceride (mg/dl)	78.97 ± 22.31	98.62 ± 39.81	95.83 ± 39.12	0.066
HDL cholesterol (mg/dl)	50.70 ± 9.06	49.17 ± 9.95	49.17 ± 12.04	0.809
LDL cholesterol (mg/dl)	101.03 ± 9.38	109.93 ± 17.92	109.86 ± 23.10	0.157
Total /HDL cholesterol	3.51 ± 0.69	3.90 ± 0.88	3.92 ± 0.97	0.120
Apo A (mg/dl)	144.70 ± 18.65	144.55 ± 21.67	144.72 ± 22.43	0.999
Apo B (mg/dl)	87.80 ± 17.42	97.53 ± 21.08	96.53 ± 20.87	0.122

The desirable level for total cholesterol is under 200 mg/dl. Cholesterol levels of 200 to 240 mg/dl are borderline high. As the results show, the mean concentrations of total cholesterol for the three groups were less than 200 mg/dl and they were not statistically significant. The mean concentrations of triglyceride for all groups were below 150 mg/dl, which is within the optimal level.

Based on the NCEP (National Cholesterol Education Program), high density lipoprotein cholesterol less than 50 mg/dl for women is low and is considered a risk factor for heart disease. HDL cholesterol values above 60 mg/dl are optimal and are considered to offer some protection against coronary heart disease. The mean concentration of HDL cholesterol in the present study was more than 50 mg/dl. There were no differences among the three groups in HDL cholesterol concentration. The value less than 100 mg/dl implies a desirable level of LDL cholesterol. The values between 100-129mg/dl are near optimal and values between 130-159 mg/dl are borderline high. The mean concentrations of LDL cholesterol for the three groups were near the optimal level.

Based on the NCEP (2005) the optimum ratio for the total to HDL cholesterol is less than 3.5, and ratio less than 4.0 is considered as low risk for women. The mean values for total cholesterol to HDL cholesterol ratio for the three groups were between 3.5 and 4.0. The ratio increases when the total cholesterol increases and HDL cholesterol decreases. Low ratio shows lower risk of heart attack, while high rate indicates higher risk.

The mean concentrations of Apo A, which manifests anti-atherogenic high density lipoprotein (HDL) particles, and Apo B, which reflects the number of potentially atherogenic lipoprotein particles, were within the normal range (80-175mg/dl and 45-120mg/dl for women, respectively). High Apo B to Apo A ratio indicates increased risk of CVD. The risk for all three groups is less than 0.8, which could be considered as a protective factor for CVD. Results of statistical tests for each lipid parameter of the subjects are presented and explained separately below.

5-3-1 Cholesterol

Changes in the mean serum cholesterol concentration of the subjects during the course of the study are shown in Table 5-3-1-1. There were no statistically significant changes in total cholesterol concentration between baseline, third and sixth weeks of the study for any group. The cholesterol concentration decreased by 4.07% after six weeks in the probiotic yoghurt group, compared to 2.83% for the conventional yoghurt group. Though there were positive changes in both yoghurt groups, the results were not statistically significant within a single group. There was a 3.79% increase in the control group.

Table 5-3-1-1 Means and standard deviations of blood total cholesterol during the study in each study group

Variable	Intervals Groups	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference ^a (%)
Total Cholesterol (mg/dl)	Conventional yoghurt	173.90±25.62	173.17±29.01	168.97±22.26	0.727	-2.83
	Probiotic yoghurt	185.66±23.86	184.41±27.98	178.10±25.42	0.493	-4.07
	Control	184.38±27.85	185.07±33.86	191.38±36.13	0.671	3.79

^a- percentage of change between baseline and 6th week

In Table 5-3-1-2 the differences in mean values between T1 and T3 interval are presented. The differences between baseline means and sixth week means for cholesterol were statistically significant among the three groups ($P=0.001$). There was a decrease in both probiotic and conventional yoghurt consuming groups and an increase in the control group. The decrease was greater for the probiotic yoghurt ($P<0.005$) than the conventional yoghurt group ($P<0.05$).

Table 5-3-1-2 Differences between baseline (T1) mean and 6th week (T3) mean of blood cholesterol for the three groups

		T1-T3 (mean ± SD)	P value
Variable	Groups		
Total Cholesterol (mg/dl)	Conventional yoghurt	-4.93 ± 13.74*	0.001
	Probiotic yoghurt	-7.56 ± 12.83**	
	Control	7.00 ± 19.85	

* $P<0.05$ vs. control, ** $P<0.005$ vs. control

The result of multiple comparisons for the three groups by Bonferroni post-hoc test is presented in Table 5-3-1-3. The decrease in mean cholesterol concentrations were statistically significant for both conventional and probiotic groups, compared to the control group, respectively, $P<0.014$ and $P<0.002$.

Table 5-3-1-3 Multiple comparisons of differences between baseline (T1) mean and 6th week (T3) mean of blood cholesterol among the three groups

Week (T3) mean of blood cholesterol among the three groups				
Variable		Intervals	T1-T3 (mean ± SE)	P value
Groups				
Cholesterol (mg/dl)	Conventional yoghurt	Probiotic yoghurt	-2.61 ± 4.10	1.000
		Control	11.93 ± 4.10*	0.014
	Probiotic yoghurt	Conventional yoghurt	2.61 ± 4.10	1.000
		Control	14.55 ± 4.14*	0.002
	Control	Conventional yoghurt	-11.93 ± 4.10*	0.014
		Probiotic yoghurt	-14.55 ± 4.14*	0.002

* The mean difference is significant at the 0.05 level.

5-3-2 Triglyceride

The means and standard deviations of triglyceride throughout the study are presented below (Table 5-3-2-1). The mean concentration of triglyceride increased in the conventional yoghurt group by 3.62% and by 7.13% in the control group. There was a 0.17% decrease in the probiotic yoghurt group. Even though there was an increase in the mean values for control and conventional yoghurt groups after six weeks, the ANOVA did not show statistically significant change throughout the study at the three intervals for any group.

Table 5-3-2-1 Means and standard deviations of triglyceride during the study in each study group

Variable	Intervals Groups	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference (%)
Triglyceride (mg/dl)	Conventional yoghurt	78.97±22.31	81.87±19.73	81.83±20.84	0.828	3.62
	Probiotic yoghurt	98.62±39.82	100.59±38.07	98.45±35.98	0.972	-0.17
	Control	95.83±39.12	100.45±31.88	102.66±33.91	0.752	7.13

Comparing the differences between baseline means and sixth week means of triglyceride among the three groups did not show any significant difference (Table 5-3-2-2). The increase in the control group was more than twice the increase in the conventional yoghurt group.

Table 5-3-2-2 Differences between baseline (T1) mean and 6th week (T3) mean of triglyceride for the three groups

Variable	Interval Groups	T1-T3 (mean ± SD)	P value
Triglyceride (mg/dl)	Conventional yoghurt	2.87 ± 11.70	0.423
	Probiotic yoghurt	-0.17 ± 26.56	
	Control	6.83 ± 20.03	

5-3-3 HDL cholesterol

The HDL cholesterol concentrations of the three groups at the three intervals of the study are presented in Table 5-3-3-1. There were improvements in HDL cholesterol concentrations in both yoghurt consuming groups. The highest increase in means from T1 to T3 was in the probiotic yoghurt consuming group - by 8.76%. The increase in the conventional yoghurt group was 5.18%. There was a decrease by 0.77% in the control group. But, by conducting the ANOVA, changes in HDL cholesterol concentration during the course of the study within a group were shown to be not significant for any group.

Table 5-3-3-1 Means and standard deviations of HDL cholesterol during the study in each study group

Variable	Intervals Groups	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference (%)
HDL Cholesterol (mg/dl)	Conventional yoghurt	50.70±9.06	54.43±8.25	53.33±8.88	0.241	5.18
	Probiotic yoghurt	49.17±9.95	52.97±8.13	53.48±9.26	0.153	8.76
	Control	49.17±12.04	50.66±13.40	48.79±12.44	0.839	-0.77

The difference between baseline mean and 6th week mean concentrations of HDL cholesterol is shown in Table 5-3-3-2. There was an improvement in HDL cholesterol concentration in both yoghurt consuming groups. The improvement in the probiotic yoghurt group was higher than the conventional yoghurt group. The ANOVA showed statistically significant differences among the three groups (P<0.05).

Table 5-3-3-2 Differences between baseline (T1) mean and 6th week (T3) mean of HDL cholesterol for the three groups

Variable	Interval Groups	T1-T3 (mean ± SD)	P value
HDL Cholesterol (mg/dl)	Conventional yoghurt	2.63 ± 6.65	0.011
	Probiotic yoghurt	4.31 ± 5.70*	
	Control	-0.38 ± 5.11	

* P<0.05 vs. control

The multiple comparisons between the groups showed that the increase in mean concentration of HDL cholesterol for the probiotic yoghurt group was statistically significant, compared to the control group ($P=0.009$). (Table 5-3-3-3) The differences between the probiotic and the conventional yoghurt groups, as well as differences between the conventional yoghurt and the control groups were not statistically significant.

Table 5-3-3-3 Multiple comparisons of differences between baseline (T1) mean and 6th week (T3) mean of HDL cholesterol among the three groups

Variable	Interval		T1-T3 (mean \pm SE)	P value
	Groups			
HDL Cholesterol (mg/dl)	Conventional yoghurt	Probiotic yoghurt	1.68 \pm 1.53	0.827
		Control	-3.01 \pm 1.53	0.156
	Probiotic yoghurt	Conventional yoghurt	-1.68 \pm 1.53	0.827
		Control	-4.69 \pm 1.54*	0.009
	Control	Conventional yoghurt	3.01 \pm 1.52	0.156
		Probiotic yoghurt	4.69 \pm 1.54*	0.009

* The mean difference is significant at the 0.05 level.

5-3-4 LDL cholesterol

Low Density lipoprotein cholesterol concentration decreased by 1.14% in the conventional yoghurt group and by 1.97% in the probiotic yoghurt group after 6 week intervention compared to the baseline values (Table 5-3-4-1). There was a 3.13% increase in LDL cholesterol concentration in the control group. The mean concentration of LDL cholesterol showed an ascending trend in the third week of intervention for the both yoghurt groups followed by a decrease in the sixth week. The ANOVA did not show any statistically significant change for LDL cholesterol concentration from baseline to third and sixth week of the study for any group.

Table 5-3-4-1 Means and standard deviations of LDL cholesterol during the study in each study group

Variable	Intervals Groups	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference (%)
LDL-C (mg/dl)	Conventional yoghurt	101.03±19.38	103.07±21.86	99.87±19.16	0.825	-1.14
	Probiotic yoghurt	109.93±17.92	110.03±20.69	107.76±20.70	0.885	-1.97
	Control	109.86±23.10	113.31±24.29	113.03±24.21	0.830	3.13

The differences between baseline and sixth week concentrations of LDL cholesterol showed improvement in values for the probiotic and the conventional yoghurt groups while there was an increase in the control group (Table 5-3-4-2). The differences between baseline and sixth week concentrations of LDL cholesterol were not statistically significant among the three groups.

Table 5-3-4-2 Differences between baseline (T1) mean and 6th week (T3) mean of LDL cholesterol for the three groups

Variable	Interval Groups	T1-T3 (mean ± SD)	P value
LDL-C (mg/dl)	Conventional yoghurt	-1.17 ± 9.36	0.243
	Probiotic yoghurt	-2.17 ± 11.27	
	Control	3.17 ± 16.66	

5-3-5 Total to HDL cholesterol ratio

The total cholesterol to HDL cholesterol ratio helps to predict an individual's risk of developing atherosclerosis. High ratios indicate higher risks of CVD, low ratios indicate lower risks. According to American Heart Association (AHA) recommendations, for women, a ratio of less than 4.0 is considered low risk and a ratio less than 5.0 is considered

average risk. The ratio was less than 4.0 for all groups, except for the control group at the third interval (4.16).

Total cholesterol to HDL cholesterol ratio for the three groups at the three intervals is presented below (Table 5-3-5-1). After six week intervention, there was a 12.56% decrease in total cholesterol to HDL cholesterol ratio in the probiotic yoghurt group and 7.69% decrease in the conventional yoghurt group. There was a 6.12% increase in the control group. The improvement in the total cholesterol to HDL cholesterol ratio was twice as much in the probiotic yoghurt group compared to the conventional yoghurt group. The ANOVA did not show any statistically significant differences among the three intervals of the study within a group. The improvement that was observed in total cholesterol to HDL cholesterol ratio in both yoghurt consuming groups is due to the decrease in total cholesterol concentration and the increase in HDL cholesterol concentration in both yoghurt consuming groups.

Table 5-3-5-1 Means and standard deviations of total/ HDL cholesterol ratio during the study in each study group

Intervals		Baseline (T1)	3 rd week (T2)	6 th week (T3)	P-value	Deviation difference (%)
Variable	Groups					
Total/HDL-Cholesterol	Conventional yoghurt	3.51 ± 0.69	3.23±0.65	3.24±0.62	0.183	-7.69
	Probiotic yoghurt	3.90 ± 0.88	3.55±0.76	3.41±0.73	0.058	-12.56
	Control	3.92 ± 0.97	3.83±1.38	4.16±1.41	0.592	6.12

The differences between baseline and 6th week mean concentrations of total to HDL cholesterol ratio were statistically significant among the three groups (P<000). (Table 5-3-5-2) The improvement in the ratio was significant for the probiotic yoghurt group at P<0.001 and for the conventional yoghurt group at P<0.05 compared to the control group.

Table 5-3-5-2 Differences between baseline (T1) mean and 6th week (T3) mean of total/ HDL cholesterol ratio for the three groups

Interval		T1-T3 (mean ± SD)	P value
Variable	Groups		
Total/HDL- Cholesterol	Conventional yoghurt	-0.27 ± 0.44*	0.0001
	Probiotic yoghurt	-0.49 ± 0.57**	
	Control	0.24 ± 0.77	

* P<0.05 vs control , ** P<0.001 vs control

Multiple comparisons of the groups by Bonferroni showed significant increases in the ratio for both conventional yoghurt (P<0.006) and probiotic yoghurt groups (P<0.0001) compared to the control (Table 5-3-5-3).

Table 5-3-5-3 Multiple comparisons of differences between baseline (T1) mean and 6th week (T3) mean of total/ HDL cholesterol among the three groups

Interval			T1-T3 (mean ± SE)	P value
Variable	Groups			
Total/HDL Cholesterol	Conventional yoghurt	Probiotic yoghurt	-0.22 ± 0.16	.483
		Control	0.51 ± 0.16*	.006
	Probiotic yoghurt	Conventional yoghurt	0.22 ± 0.16	.483
		Control	0.73 ± 0.16*	.000
	Control	Conventional yoghurt	-0.51 ± 0.156*	.006
		Probiotic yoghurt	-0.73 ± 0.16*	.000

* The mean difference is significant at the 0.05 level.

5-3-6 Apolipoprotein A

Apolipoprotein A values for each study group throughout the intervention are presented in Table 5-3-6-1. Comparing the mean concentrations of Apo A at the three intervals by carrying out the ANOVA, did not show any statistically significant changes during the study for any group. There was an ascending trend in Apo A concentrations within the three

groups. The highest increase was in the probiotic yoghurt group by 5.61% followed by conventional yoghurt by 3.15%.

Epidemiological studies have shown that plasma Apo A concentrations like those of HDL cholesterol are inversely related to the CVD (Walldius and Jungner, 2004). Apolipoprotein A is the main apolipoprotein in HDL cholesterol. The trend that was observed in the change in HDL cholesterol concentration in both yoghurt groups throughout the study is similar to what was observed for Apolipoprotein A. There was an improvement in HDL cholesterol concentration in both yoghurt groups, with a higher increase in the probiotic yoghurt group. Here, too, there was an increase in Apolipoprotein A in both yoghurt groups, with the highest increase in the probiotic yoghurt group.

Table 5-3-6-1 Means and standard deviations of Apo A during the study in each study group

Variable	Intervals	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P-value	Deviation difference (%)
	Groups					
ApoA (mg/dl)	Conventional yoghurt	144.70±18.65	151.30±21.27	149.27±20.01	0.428	3.15
	Probiotic yoghurt	144.55±21.67	152.00±19.53	152.66±20.72	0.258	5.61
	Control	144.72±22.43	147.00±19.76	146.70±20.79	0.906	1.36

The ANOVA showed no statistically significant differences between baseline and sixth week mean concentrations of Apo A among the three groups, as illustrated in Table 5-3-6-2. The highest increase in Apo A concentration was observed in the probiotic yoghurt group followed by the conventional yoghurt group.

Table 5-3-6-2 Differences between baseline (T1) mean and 6th week (T3) mean of Apo A among the three groups

Variable	Interval	T1-T3 (mean ± SD)	P value
	Groups		
Apo A (mg/dl)	Conventional yoghurt	4.57 ± 16.33	0.307
	Probiotic yoghurt	8.10 ± 16.85	
	Control	1.98 ± 11.68	

5-3-7 Apolipoprotein B

The means and standard deviations of Apolipoprotein B at the three intervals of the study for each study group are demonstrated in Table 5-3-7-1. The values were almost constant in both of the yoghurt groups, but there was an ascending trend in the control group. The changes in mean values at the three intervals were not statistically significant for any group (Table 5-3-7-1).

Apolipoprotein B is the primary Apolipoprotein of low density lipoprotein cholesterol which carries cholesterol to tissues. Although LDL cholesterol is commonly used to assess CVD risk, Apo B may better reflect the risk. LDL cholesterol levels can be inaccurate in individuals with kidney and liver diseases, high triglyceride, and diabetes mellitus (Walldius and Jungner, 2004). In individuals who have normal cholesterol and triglyceride levels, Apo B assessment can identify those at higher risk. This is because it identifies the risk-increasing small and dense LDL particles.

Table 5-3-7-1 Means and standard deviations of Apo B during the study in each study group

Variable	Intervals	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P-value	Deviation difference (%)
	Groups					
Apo B (mg/dl)	Conventional yoghurt	87.80 ± 17.42	89.95±21.65	87.67±19.11	0.879	-0.15
	Probiotic yoghurt	97.53 ± 21.08	96.62±17.65	97.43±19.61	0.981	-0.10
	Control	96.53 ± 20.87	98.61±20.75	99.54±20.77	0.854	3.12

An elevated Apo B to Apo A ratio could be a risk factor for CVD. The ratio less than 0.8 are associated with lower risk of CVD. The Apo B to Apo A ratio in the three intervals and in the three groups was less than 0.7.

The differences between baseline and 6th week mean values of Apo B concentrations are presented in Table 5-3-7-2. There was a decrease for conventional and probiotic yoghurt group and increase in the control group. There were no statistically significant differences among the three groups.

Table 5-3-7-2 Differences between baseline (T1) mean and 6th week (T3) mean of Apo B among the three groups

Interval		T1-T3 (mean ± SD)	P value
Variable	Groups		
Apo B (mg/dl)	Conventional yoghurt	-0.13 ± 9.75	0.456
	Probiotic yoghurt	-0.10 ± 12.21	
	Control	3.01 ± 10.70	

Table 5-3-7-3 shows the correlation between mean differences of total/HDL cholesterol ratio (T1 - T3) and mean differences (T1 - T3) of total cholesterol, and HDL cholesterol in the three groups. There was a positive and significant correlation between mean difference of total/HDL cholesterol ratio and mean difference of total cholesterol in both probiotic and control groups ($P < 0.05$ and $P < 0.01$). The correlation was negative and significant for HDL cholesterol mean differences in all three groups ($P < 0.01$).

Table 5-3-7-3 Correlation between mean differences (T1-T3) of total/HDL cholesterol ratio and lipid parameters in the three groups

Variables		Correlation	r	P
Groups				
Total cholesterol mean difference (T1-T3)	Conventional yoghurt		0.333	NS*
	Probiotic yoghurt		0.404	<0.05
	Control		0.611	<0.01
HDL cholesterol mean difference (T1-T3)	Conventional yoghurt		-0.645	<0.01
	Probiotic yoghurt		-0.764	<0.01
	Control		-0.670	<0.01

NS: not significant

5-3-8 Summary of results for blood lipid parameters

There were no statistically significant differences among the three groups in blood lipid parameters at baseline. There were no statistically significant differences throughout the study for lipid parameters within any group. Only in probiotic yoghurt group was the decline in total/HDL cholesterol ratio of 12.56% ($P = 0.058$).

Comparing the mean differences between baseline and six week interval for triglyceride, LDL cholesterol, Apo A and Apo B showed no statistically significant differences among the three groups. For cholesterol, the differences were significant for both conventional and probiotic yoghurt groups at $P<0.05$ and $P<0.005$, respectively.

For total to HDL cholesterol ratio the differences were also significant for both conventional and probiotic yoghurt groups at $P<0.05$ and $P<0.001$ respectively. The differences in mean concentrations of HDL cholesterol were significant for probiotic yoghurt group only ($P<0.01$).

There was a positive and significant correlation between mean difference of total/HDL cholesterol ratio and mean difference of total cholesterol in both probiotic and control groups ($P<0.05$ and $P<0.01$). The correlation was negative and significant for HDL cholesterol mean differences in all three groups ($P<0.01$).

5-4 Oxidative stress parameters

Means and standard deviations of total antioxidant capacity (TAC), oxidized LDL, and malondialdehyde (MDA) at baseline are presented in Table 5-4-1. The ANOVA was applied to identify any differences in total antioxidant capacity, and oxidized LDL, among the three groups at baseline. Kolmogorov-Smirnov test was conducted. The distribution of MDA was not normal. Napierian logarithm (i.e., the logarithm to the base e) was applied to convert MDA values to normal distribution. The ANOVA test did not show any statistically significant difference among the three groups at baseline for any of these parameters.

Table 5-4-1 Means and standard deviations of blood oxidant and antioxidant parameters of subjects at baseline

Variables \ Groups	Conventional yoghurt	Probiotic yoghurt	Control	P- value
TAC (mmol/l)	3.76 ± 0.28	3.74 ± 0.30	3.62 ± 0.35	0.182
Oxidized LDL (U/l)	72.80 ± 21.66	81.56 ± 21.65	84.89 ± 20.17	0.082
MDA (nmol/ml) ^a	2.49 ± 1.23	2.61 ± 1.20	2.65 ± 1.23	0.453

^a Geometric mean is presented for MDA

5-4-1 Total Antioxidant Capacity (TAC)

Means and standard deviations of TAC throughout the study for the three groups are presented in Table 5-4-1-1. To identify any change throughout the study at the three intervals, the ANOVA was used. The decreases of TAC values in the conventional and the probiotic yoghurt groups at third (T2) and sixth week (T3) of study were statistically significant at $P < 0.0001$, compared to baseline values. There was a 14.36% decrease in TAC values for the conventional yoghurt group, and a 10.42% decrease in the probiotic yoghurt group. The decrease for the control group was 5.80%, which was half the value for the probiotic yoghurt and one third of the value for the conventional yoghurt.

Table 5-4-1-1 Means and standard deviations of TAC during the study for the three groups

Variable	Intervals Groups	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference (%)
TAC (mmol/l)	Conventional yoghurt	3.76 ± 0.28	3.45 ± 0.41^a	3.23 ± 0.41^b	0.0001	-14.36
	Probiotic yoghurt	3.74 ± 0.30	3.51 ± 0.35^c	3.36 ± 0.37^b	0.0001	-10.42
	No yoghurt	3.62 ± 0.35	3.53 ± 0.30	3.41 ± 0.36	0.064	-5.80

^a $P < 0.01$ vs. baseline, ^b $P < 0.0001$ vs. baseline, ^c $P < 0.05$ vs. baseline

The difference between baseline and sixth week means for each group is presented in Table 5-4-1-2. The ANOVA showed statistically significant difference between baseline and sixth week means for the conventional yoghurt group compared to the control group. ($P = 0.002$)

Table 5-4-1-2 Differences between baseline (T1) mean and 6th week (T3) mean of TAC for the three groups

Variable	Interval Groups	T1-T3 (mean \pm SD)	P value
TAC (mmol/l)	Conventional yoghurt	$-0.54 \pm 0.36^*$	0.002
	Probiotic yoghurt	-0.39 ± 0.30	
	Control	-0.21 ± 0.37	

* $P = 0.001$ vs. control

Multiple comparisons of mean differences between T1 and T3 intervals, by conducting Bonferroni post-hoc test, showed significant difference between the conventional yoghurt and the control groups ($P=0.001$). (Table 5-4-1-3)

Table 5-4-1-3 Multiple comparisons of difference between baseline (T1) mean and 6th week (T3) mean of TAC for the three groups

Variable	Intervals		T1-T3 (mean \pm SE)	P value
	Groups			
TAC (mmol/l)	Conventional yoghurt	Probiotic yoghurt	0.15 \pm 0.09	0.292
		Control	0.33 \pm 0.09*	0.001
	Probiotic yoghurt	Conventional yoghurt	-0.15 \pm 0.09	0.292
		Control	0.17 \pm 0.09	0.166
	Control	Conventional yoghurt	-0.33 \pm 0.09*	0.001
		Probiotic yoghurt	-0.17 \pm 0.09	0.166

* The mean difference is significant at the 0.05 level.

5-4-2 Oxidized LDL

The means and standard deviations of oxidized LDL during the study are illustrated below (Table 5-4-2-1). There was a 3.71% increase in oxidized LDL in the probiotic yoghurt group, and a 2.18% increase in the conventional yoghurt group after 6-week intervention. There was a 0.73% decrease in the control group. Although there was an ascending trend in oxidized LDL in both yoghurt groups, the ANOVA did not show any statistically significant differences in oxidized LDL values in the three intervals of the study in any group.

Table 5-4-2-1 Means and standard deviations of oxidized LDL during the study for the three groups

Variable	Groups	Interval			P-value	Deviation difference (%)
		Baseline (T1)	3 rd week (T2)	6 th week (T3)		
Oxidized LDL (U/l)	Conventional yoghurt	72.80 \pm 21.66	73.86 \pm 23.78	74.39 \pm 23.64	0.963	2.18
	Probiotic yoghurt	81.56 \pm 21.65	83.15 \pm 23.20	84.59 \pm 23.94	0.882	3.71
	Control	84.89 \pm 20.17	82.98 \pm 21.73	84.27 \pm 20.80	0.939	-0.73

The ANOVA did not show any difference among the three groups in terms of difference between baseline (T1) and sixth week (T3) mean concentration of oxidized LDL (Table 5-4-2-2). The highest increase in the oxidized LDL value was in the probiotic yoghurt group.

Table 5-4-2-2 Differences between baseline (T1) mean and 6th week (T3) mean of oxidized LDL for the three groups

Variable	Interval	T1-T3 (mean \pm SD)	P value
	Groups		
Oxidized LDL (U/l)	Conventional yoghurt	1.60 \pm 8.42	0.328
	Probiotic yoghurt	3.02 \pm 9.31	
	Control	-0.61 \pm 10.06	

5-4-3 Malondialdehyde (MDA)

The distribution of MDA was not normal; a Naperian log transformation of MDA values was performed to make data fit the normal distribution. To identify any differences in MDA values in the three intervals, the ANOVA was applied. Table 5-4-3-1 shows the geometric means of MDA, at the three intervals, for each study group.

There was a 4.2% increase in MDA mean values in the probiotic yoghurt group and a 2.40% increase in the conventional yoghurt group, after six weeks. In the control group a 5.28% decrease in the MDA mean values was observed. The ANOVA did not show any significant differences in the intervals of the study for MDA in any group.

Table 5-4-3-1 Geometric means and standard deviations of MDA during the study for the three groups

Variable	Intervals	Baseline (T1)	3 rd week (T2)	6 th week (T3)	P- value	Deviation difference (%)
	Groups					
MDA (nmol/ml)	Conventional yoghurt	2.50 \pm 1.23	2.50 \pm 1.21	2.55 \pm 1.24	0.898	2.40
	Probiotic yoghurt	2.62 \pm 1.20	2.58 \pm 1.27	2.72 \pm 1.22	0.567	4.20
	Control	2.66 \pm 1.23	2.49 \pm 1.24	2.52 \pm 1.27	0.511	-5.28

Differences between baseline (T1) and sixth week (T3) geometric means of MDA for each group are presented in Table 5-4-3-2. The ANOVA showed statistically significant difference in geometric means of MDA among the three groups. The difference was only significant for the probiotic yoghurt group compared to the control group (P=0.030).

Table 5-4-3-2 Differences between baseline (T1) and 6th week (T3) geometric mean of MDA for the three groups

Interval		T1-T3 (mean ± SD)	P value
Variable	Groups		
MDA (nmol/ml)	Conventional yoghurt	0.98 ± 1.13	0.030
	Probiotic yoghurt	0.96 ± 1.14*	
	Control	-1.05 ± 1.17	

* P<0.05 vs. control

Multiple comparisons of three groups showed significant differences in MDA values for the probiotic yoghurt group compared to the control group (P=0.038) (Table 5-4-3-3).

Table 5-4-3-3 Multiple comparisons of differences between baseline (T1) and 6th week (T3) geometric means of MDA for the three groups

Intervals			T1-T3 (mean ± SE)	P value
Variable	Groups			
MDA (nmol/ml)	Conventional yoghurt	Probiotic yoghurt	1.02 ± 1.04	1.000
		Control	0.92 ± 1.04	0.125
	Probiotic yoghurt	Conventional yoghurt	0.98 ± 1.04	1.000
		Control	0.91 ± 1.04*	0.038
	Control	Conventional yoghurt	1.08 ± 1.04	0.125
		Probiotic yoghurt	1.10 ± 1.04*	0.038

* The mean difference is significant at the 0.05 level

5-4-4 Summary of results for oxidant and antioxidant parameters

The ANOVA results showed no statistically significant differences among the three groups at baseline for TAC, oxidized LDL, and MDA concentrations in the blood.

TAC was significantly lower in third week (T2) and sixth week (T3) of the study, in both conventional (T2<T1; $P<0.01$ & T3<T1; $P<0.0001$) and probiotic yoghurt groups (T2<T1; $P<0.05$ & T3<T1; $P<0.0001$) compared to the control group. The ANOVA also, showed statistically significant differences between baseline and sixth week mean values of TAC for the conventional yoghurt group compared to the control group ($P=0.002$).

There were no statistically significant differences in oxidized LDL among the three intervals of the study within any group. Neither were any differences between baseline and sixth week values among the three groups.

For MDA, the differences between the three intervals were not statistically significant, but the changes between baseline and sixth week values were statistically higher for the probiotic group ($P=0.038$) compared to the control.

6 DISCUSSION

6-1 General characteristics of the subjects

There were no differences in age, body weight, height and BMI of the subjects at the beginning of the study among the three groups. The mean values for age, body weight and BMI tended to be a little higher in the probiotic yoghurt group compared to the other two groups, but they were not statistically significant. More importantly, comparing body weight and BMI of the three groups at the three intervals, showed almost no change in these parameters in any group. In fact, the P- values were either one or very close to one for all the three groups. The mean value for BMI was in normal range for three groups throughout the study. Changes in weight and BMI are a very important concern, especially in studies on lipid profile; changes in weight and BMI during the course of intervention can independently influence the results and can act as a confounding factor.

One of the early studies on the effect of milk on cholesterol concentrations was done on the Maasai people in Africa (Mann and Spoerry, 1974). The study came to an end after 3 week instead of 4 week. The reason for this was the great quantity of milk drunk by the participants. The researchers prompted subjects to exercise more. However, subjects did not implement any increase in their exercise and therefore put on weight. In case of the Maasai tribe, even though the subjects put on weight, there was a considerable reduction in cholesterol concentrations in both fermented milk consuming groups.

In another trial on the effect of a fermented milk product *GAIO* on risk factors for cardiovascular diseases in obese and overweight subjects, when comparing all experimental groups, without adjustment for alterations in body weight, no statistically significant effect was seen in LDL cholesterol in the probiotic group. The reduction in LDL cholesterol (8.4%) and increase in fibrinogen happened in probiotic (*GAIO*) consuming group compared to the acidified milk consuming group, only after applying adjustment for body weight alteration (Agerholm-Larsen et al, 2000).

6-2 Dietary intakes of the subjects

In some studies, researchers gave the subject the probiotic bacteria in the form of a capsule. The reason for this was to simplify the distribution process and also, to do away with

obliging participants to alter their eating habits or eat research- connected foods. Adding dairy products to the food regime as a means of providing probiotics can change the dietary intakes of the subjects. As a result of this change, the energy, fat, and some other nutrient intakes of the subjects may be altered. The change in nutrient intakes can influence the results of a study and act as a confounding factor. Nevertheless a dairy product seems to be the most effective vehicle for giving probiotic bacteria to people. Most of the research that utilized fermented milk, in the form of yogurt or dairy products, showed an hypocholesterolemic influence (Agerback et al, 1995; Bertolami et al, 1999; Schaafsma et al, 1998; Xiao et al, 2003). This is in comparison to the failure of placebo-controlled studies that fed non dairy food (Jahreis et al, 2002) or capsules to participants (Lewis and Burmeister, 2005; Greany et al, 2008). Controlling the energy and fat intake, therefore, is of great importance in such studies.

Greany et al (Greany et al, 2004) conducted a study to assess the independent and synergetic impact of soy and probiotic bacteria on some lipid parameters in humans. The study was done on postmenopausal females, who were assembled into four intervention groups: Of the four groups, two of them consumed soy protein (one soy protein alone, the other with probiotic capsules). The other two groups were given milk protein (again one of them milk protein alone, the other with probiotic capsules). The nutrient ingestion at baseline and during the soy and milk regime interval was compared. The evaluation indicated a reduction in the amount of fiber and protein consumed ($P < 0.004$ & $P < 0.0001$). On the other hand, no notable change was seen in the amount of energy or macronutrients consumed in their regime. No adjustment was applied. According to the study, probiotics did not boost the effects of soy. Nor did it help to reduce cholesterol.

In a trial conducted by Agerholm-Larsen et al (Agerholm-Larsen et al, 2000), four out of five groups were given yogurt with various strains of bacteria, whereas the remaining group was given placebos. The duration of the intervention was 8 weeks and subjects were required to fill out 7- day records of their food intake at the start and the end of the study. The weighted records did not show any difference in average energy consumption across the different groups. The same was true for macronutrient intake. Changes in intake of alcohol and carbohydrates from baseline to 8-week, however, were significant among the groups. The study stated that usual individual differences and multiple testing could

account for the dissimilarity seen between the groups. When statistically weight gain (changes) was controlled in the probiotic group, there was a reduction in LDL cholesterol.

A cross-over study was conducted to examine the effect of yogurt with *L.acidophilus* on plasma cholesterol. Participants with elevated cholesterol levels had to take 200 ml fermented milk for the course of 3 weeks (Anderson and Gilliland, 1999). Participants were asked to maintain their normal diet for the course of the intervention. Also, they were required to fill out three-day diet records and turn them in at each interval and at the end of each fermented milk treatment. No meaningful difference in terms of nutrient ingestion was shown in the mean dietary consumption (proteins, energy, carbohydrates, cholesterol, fatty acids and alcohol) between the groups or during the trial period. The results showed a reduction in cholesterol concentration in the probiotic consuming group.

In a cross-over study by Fabian and Elmadfa (Fabian and Elmadfa, 2006), volunteers consumed 100 g/day conventional or probiotic yoghurt for 2 weeks and 200 g/day for another 2 weeks. Means for dietary intakes were calculated by 24 hour food records. There were no changes in dietary intakes within or between the tested groups throughout the investigation. Consumption of both conventional and probiotic yoghurts for 4 weeks had a positive effect on the lipid profile in the plasma of healthy women.

There is a possibility that by introducing yoghurt to the diet of subjects, a change in fat intake or consequently weight gain may occur. We did not find any significant changes in energy, fat and relevant nutrient intakes within or among the groups during the study. At the three intervals (baseline, 3 week, 6 week) three-day dietary records were taken from each volunteer who was directed to write down the types and amounts of foods eaten. The amount of food eaten by each subjects was estimated from household measures and from photos of serving sizes. A nutritionist checked the participants' dietary records in person upon their periodically visits to the Nutrition Department at TUMS. Subjects were instructed to maintain their usual dietary habits throughout the experiment. Weekly follow ups were performed by phone. Monitoring the diets of the participants helped to avoid any changes in their diets. Furthermore, consumption of yoghurt is part of a dietary habit in Iran. Therefore, supplementing the yoghurt in the diet of the subjects did not cause a modification in their dietary habits.

6-3 The effects of probiotic yoghurt on blood lipid parameters

Hypothesis: There are improvements in lipid profile parameters in probiotic and conventional yoghurt consuming groups compared to the control group.

In the present study, at baseline, there were not statistically significant differences among the three groups in total cholesterol, triglyceride, HDL and LDL cholesterol, total/HDL cholesterol ratio, Apo A and Apo B concentrations.

There were descending trends in total cholesterol and LDL cholesterol concentrations throughout the study in both conventional yoghurt (-2.83% and -1.14%) and probiotic yoghurt (-4.07% and -1.97%) groups. There was an ascending trend in HDL cholesterol concentration throughout the study in the conventional (5.18) and the probiotic (8.78) yoghurt consuming groups. An ascending trend was also seen in Apo-A concentration in both yoghurt groups. Despite all these alterations in lipid profile, there were no statistically significant differences within group for any of these parameters. The significant results were obtained only when mean differences (T1-T3) were compared among the three groups.

Comparing changes in the mean differences (T1-T3) showed significant improvement in total cholesterol and total to HDL cholesterol ratio in both conventional and probiotic yoghurt groups compared to the control. The improvement in mean differences (T1-T3) of HDL cholesterol was only significant for the probiotic yoghurt consuming group compared to the control. The positive and significant changes that were observed in this study were attributed to both the conventional and probiotic yoghurt groups. There were no differences between the two yoghurt consuming groups.

The reason that we did not observe any significant improvement in lipid parameters within a group could be that our subjects in all three groups were healthy people and had low risk of cardiovascular disease. As presented in the results section, all the subjects at baseline and throughout the study had HDL cholesterol values above 60 mg/dl. This could offer some protection against coronary heart disease. The optimum ratio for the total to HDL cholesterol is less than 3.5, and ratio less than 4.0 is considered as low risk for women. The mean values for total cholesterol to HDL cholesterol ratio for the three groups were between 3.5 and 4.0. High Apo B to Apo A ratio indicates increased risk of CVD. The risk for all

three groups was less than 0.8, which could be considered as a protective factor for CVD. In addition, the dietary intakes of the subjects also could have protective effect. In the present study, the mean values for SFA, MUFA, and PUFA from total energy were between 10 to 11 percent in the three groups (Table 5-2-1). It is suggested that the polyunsaturated to saturated fatty acids ratio close to one could lower the risk of cardiovascular diseases. In the present study, at baseline, the P/S ratio was 1.03 for both yoghurt groups and 0.95 for the control group. The dietary intakes of the subjects, too, could have a protective effect and could be the reason that significant changes were not observed within a group.

Consumption of fermented milk (yoghurt) could account for the reduction in cholesterol concentration in this study. Some early studies on fermented milk (FM) also showed hypocholesterolemic effect of these products on lipid plasma. Human studies on effect of fermented dairy products on cholesterolemia have been conducted since the 1970s. In an experiment in which 4 groups supplemented their diet with unpasteurized yoghurt, pasteurized yoghurt, or 2%-fat milk, or continued their regular diet for 3 months, cholesterol decreased by 9% in the group consuming unpasteurized yoghurt, after the first week of the intervention (Hepner et al, 1979). In the yoghurt consuming groups the decrease was 5%. The researchers concluded that a minor cholesterol lowering effect may possibly be contributed to milk. They also concluded that yoghurts (pasteurized and not pasteurized) had the same effect on levels of cholesterol.

Harrison and Peat (Harrison and Peat, 1975) demonstrated that in comparison to the control formula, infant formula containing *L. acidophilus* caused lower serum cholesterol amounts. In a study by Agerbaek et al (Agerbaeck et al, 1995), examining the relationship between *Enterococcus faecium* fermented milk products plus two strains of *S. thermophilus*, on lipid profile; a meaningful decrease of 10 % in low density cholesterol was discovered after daily intake of fermented milk products for six weeks. This was in comparison to control group which consumed acidified milk. High density lipoprotein and triglyceride remained constant in the two groups. After a six-month trial, the decrease in low density lipoprotein level of the subjects was the same as that of those that took the placebo product. This shows the probable existence of cholesterol lowering effects in both low-fat and fermented milk (Richelsen et al, 1996).

In a study conducted by Schaafsma et al, (Schaafsma et al, 1998) in which for 21 days subjects consumed milk fermented with two strains of *L. acidophilus* on a daily basis, a meaningful decrease of 4.4 % in plasma cholesterol and a 5.4 % decrease in low density lipoprotein was observed. The yogurt consumed contained a percentage of fructo-oligosaccharides (2.5 %). This could also have had an influence on level of plasma cholesterol. Concentrations of plasma high density lipid stayed unaffected. This was also true of triglyceride levels.

In a different double blind study, conducted by Anderson and Gilliland (Anderson and Gilliland, 1999), which was cross over trial and used placebos, one group consumed fermented milk with *L. acidophilus* L1, while the other was given the placebo, both for duration of four weeks. Within the first treatment period, a 3.2 percent reduction in plasma cholesterol levels was seen. During the second treatment period, neither of the groups showed any significant variation in their plasma cholesterol levels.

Akalin et al (Akalin et al, 1997) carried out a study on mice to investigate the effect of acidophilus and dietary yogurt on lipid and triglyceride levels. Besides the control group, the other two groups were given either acidophilus or ordinary yogurt. There was a significant reduction of total cholesterol by 17% and low density lipoprotein levels by 33% in the acidophil group. However, the decrease in total cholesterol and low density lipoprotein cholesterol of respectively 7% and 11% in the ordinary yogurt group was not significant. In neither group was there any change in low density lipoprotein and triglyceride levels. The trial showed that *L. acidophilus* was able to establish itself in the animal's GI tract easier than the other strain. Compared to conventional yogurt, acidophilus yogurt was capable of decreasing plasma cholesterol levels with more success.

The significant decrease in the mean difference of total to HDL cholesterol ratio in both yoghurt consuming groups in the present study could be the consequence of the decrease in total cholesterol level as well as the increase in HDL cholesterol concentration after consuming both conventional and probiotic yoghurts for 6 weeks. Kiessling et al (Kiessling et al, 2002) demonstrated that the intake of yogurt with 3.5% fat and cultures of *L. lactis* and *S.thermophilus* did not change the mean values of total or low density lipid cholesterol. This was also true for consumption of B. Longum 913, *L. acidophilus* 145, and 1% oligofructose enriched probiotic yogurt. Continued intake of these two yogurts for a twenty one week

period resulted in elevation of plasma high density lipoprotein. This in turn led to more favorable LDL to HDL ratios. The researchers proposed that cause of the increased high density lipoprotein concentrations could be the sphingolipids present in the high fat fermented milk.

In a cross-over clinical study on women with normal cholesterol levels, effects of eating probiotic yoghurt with *Lactobacillus casei subsp. casei* or conventional yoghurt were compared (Fabian and Elmadfa, 2006). There were a number of favorable changes in lipid levels of the participants, but no significant difference was seen in triglyceride, low density lipid, high density lipid, and total cholesterol levels between the probiotic and conventional yoghurt groups. The researchers' conclusion was that the change in lipid levels could probably be explained by the type and amount of fatty acids in the milk fat and the sphingolipids in the yoghurt.

In the present study, too, the effects were observed in both yoghurt consuming groups compared to the control. There were no differences between the two yoghurt groups. Another explanation for the observed effects in both yoghurt groups in this study could be the sphingolipids in yoghurt and distribution of the milk fat (Kiessling et al, 2002; Samuelson et al, 2001; Fabian and Elmadfa, 2006; Vesper et al, 1999). The conventional and the probiotic yoghurts which were used in the present study contained 2.5% fat. Therefore, the sphingolipids in both yoghurts could have been similar. However, sphingolipids also can be found in cell membranes of bacteria which were higher in probiotic yoghurt. A number of studies have demonstrated that sphingolipids can reduce LDL and total cholesterol, and are able to increase HDL cholesterol (Vesper et al, 1999; Kobayashi et al, 1997; Imaizumi et al, 1992). Other has demonstrated that sphingolipids are able to reduce the LDL/HDL ratio (Smedman et al, 1999). Complex sphingolipids and their derivatives products in the body, such as sphingosines and ceramides, are very bioactive substances that significantly impact cell regulation. Sphingolipids can be found in cell membranes, low density lipids, and compounds which contain high lipid. Cholesterol circulation and its movements from cellular tissues, and also its metabolism are affected by sphingolipids (Jian et al, 1997; Yancey et al, 1995; Zhao et al, 1996). So are its change to other products, such as bile and cholesterol esters (Boldin and Jonas, 1996). HMG-CoA reductase regulation, is another cholesterol activities that sphingomyelin affects (Gupta and Rudney, 1991). Sphingolipids' effect in lowering serum cholesterol has been demonstrated

in several long and short term studies on rats (Imaizumi et al, 1992; Kobayashi et al, 1997). The animals that were given semi-purified food enriched with sphingomyelin and glycosphingolipids, along with 4% soybean oil showed a 30% reduction in their serum total cholesterol, compared to rats that had 5% soybean oil. Their triglycerides showed no difference.

Another possible explanation for the hypocholesterolemic effects in both the probiotic and conventional yoghurt consuming groups in the present study could be the fat distribution in milk. Scientists have proposed that in different populations consumption of fatty acids or saturated fat may have a significant effect on increasing cholesterol levels and on mortality rate from cardiovascular diseases (Artauld-Wild et al, 1993). However, studies done within a population have not always shown a significant association between consumption of high fat milk or butter fat and cardiovascular disease (Ascherio & Willett, 1995; Pietinen et al, 1997). In epidemiological studies, it is hard to find meaningful association between fat intake and lipid profile in plasma within a population. This is despite the fact that significant correlation has been observed in some clinical trials between SFA and ApoB and lipid profile (Katan et al, 1995). Flaws in dietary measurements, genetic differences among the subjects in their reaction to changes in diet, differences in daily food consumption, and inappropriate sample size could all have contributed to this lack of agreement among study results. Yet another explanation could have to do with the intricate association that exists between lipid profile and food intake.

Number of extensive investigations done on diet and serum lipids failed to demonstrate a meaningful correlation the fat consumed and plasma lipids (Glueck et al, 1982; Gordon et al, 1982; Schwartz et al, 1982). In a study on children in Finland by Moilanen et al., a small, albeit weak, association was observed between Myristic Acid (C14:0) in plasma cholesterol esters and plasma cholesterol and Apo B levels (Moilanen et al, 1986). Fatty acids such as (C12:0 and C14:0) in milk are believed by other researchers to be responsible for the elevation of high density lipids levels and the reduction in TC/HDL-C ratio (Samuelson et al, 2001; Mensink et al, 2003; Temme et al, 1996).

A reverse meaningful association between short chain fatty acids in milk and plasma cholesterol was also observed in another study in older men. Such inverse correlation was

present between their waist measurements, body mass index, LDL/HDL ratios and milk (Smedman et al, 1999).

In addition, in some studies in which the probiotic was given to the subject, not in the form of dairy products containing probiotics but rather in the form of capsules, the probiotic bacteria did not show cholesterol-lowering ability (Lewis and Burmeister, 2005; Greany et al, 2008). In a pilot study, subjects were given *L. acidophilus* and *L. bulgaricus* pills for a duration of 16 weeks. This study was not placebo controlled. The results showed that the serum cholesterol had decreased in the test group. In the second crossover study, with a double blind and randomized design, subjects with high cholesterol levels were selected (Lewis and Burmeister, 2005). For six weeks the subjects had to consume freeze dried *L. acidophilus* or placebo capsules three times each day. The type of capsules volunteers had to consume was assigned randomly. No changes were noted in the serum lipids of the subjects, this is in spite of the *in vitro* capacity of *L. acidophilus* to decrease cholesterol.

In a different study, but also using placebo, normocholesterolemic participants, were given *Lactobacillus acidophilus* and *Bifidobacterium longum* plus fructo- oligosaccharide probiotic tablets or placebo tablets. Subjects had to consume these tablets for two months after which the results showed that consumption of the probiotic and placebo capsules had resulted in no change in plasma levels of total cholesterol and high and low density lipoprotein cholesterol. No change was seen in triglyceride levels as well (Greany et al, 2008).

The above mentioned studies all support the idea that fermented milk is a suitable vehicle for feeding probiotics to individuals. Based on their research on Maasai tribe, Mann and Spoerry asserted that there is a factor in milk that causes reduction in cholesterol levels. Therefore, the more milk consumption, the larger the drop in cholesterol level (Mann and Spoerry, 1974). They concluded that milk reduced cholesterol synthesis. This seems to be the general conclusion in other studies as well that milk, with whatever fat content, is effective in reducing plasma cholesterol levels (Buonopane et al, 1992; Golay et al, 1990; Sharpe et al, 1994). Different studies suggest different factors for this phenomenon. Most of the studies, however, have not been able to specifically confirm that particular component, such as riboflavin, IgG, hydroxyl-methyl glutarate, magnesium, or other compounds may be responsible for cholesterol lowering effect of milk or that it is the microflora in the GI tract that may be behind this effect.

The bacteria in fermented milk reduce lipid levels in the body through a variety of mechanisms. Taurine and glycine get conjugated with bile acids that hepatic cells create from cholesterol. As the conjugated acids pass through the small intestine, they are assimilated and led to the liver. But, as they are being reabsorbed, these acids face the intestine's flora. *Bifidobacteri*, *lactobacilli*, and other bacteria contained in fermented food hydrolyze the bile acids. Certain lactic acid bacteria strains, like *L. acidophilus*, are able to remove cholesterol from lab media and turn it into bile acids under anaerobic conditions and in the presence of bile (Gilliland, 1990; Delmi-Bouras, 2006). In human and animal trials, the reason for removal of cholesterol is that while they are in the large intestine, deconjugated bile acids do not get reabsorbed. They leave the body with urine and feces. This results in reduced recycling of bile acids and leads to an increase in production of new bile acids by the body.

Gilliland et al showed that some strains of *L. acidophilus* grown with the presence of bile have shown to have the ability to change cholesterol metabolism (Gilliland et al, 1985). In their study, the amount of bile present in the environment did not go beyond that in human intestine. One may, therefore, conclude that this could also take place in vivo. The bacteria's action, then, would leave little cholesterol to be absorbed into the blood.

Bacteria in the large intestine are active in fermenting carbohydrates and other unabsorbed polysaccharides, as well as mucus, to create short chain fatty acids (Wolever et al, 1996). The production of cholesterol is probably affected by the relative amounts of butyrate, propionate, and acetate that are created by the bacteria (Venter et al, 1990). All substrates are involved in the production of acetate, but the quantity of propionate and butyrate is not constant. Recorded changes in short chain fatty acid production in the colon is evidence of the mentioned fermentation (Wong et al, 2006). In the present study, the SCFA production was not measured in the feces.

The large intestine readily absorbs short chain fatty acids when they are in the gut. Then, the liver metabolizes them (Cummings and Macfarlane, 1997). In order to find out how much of the acetate that is created in the colon actually ends up in blood circulation, Wolever et al. studied the interaction between short chain fatty acids in the colon and in blood serum. Their study showed that combinations of sodium acetate or sodium propionate in the rectum elevated concentrations of both in the blood. Based on their findings, acetate causes an

increase in total cholesterol, and reduces fatty acids. They further concluded that propionate elevates blood glucose and reduces the cholesterol lowering response stimulated by acetate (Wolever et al, 1991).

One may presume, therefore, that consumption of dairy products fermented with the proper strain of bacteria can lead to a lower cholesterol level in blood. This study showed that both yoghurt products had a positive effect on the lipid profile of the subjects. The modulation in lipid profiles in this study could be the results of different mechanisms that were discussed as well as the fatty acid distribution of the milk fat and the sphingolipids contained in the yoghurt.

6-4 The effects of probiotic yoghurt on blood oxidant and antioxidant parameters

Hypothesis: There is a decrease in malondialdehyde and oxidized LDL concentration and an increase in total antioxidant capacity in the probiotic and conventional yoghurt groups compared to the control group.

The results of the present study showed that TAC decreased significantly in both the conventional and the probiotic yoghurt consuming groups compared to the control. The oxidized LDL and MDA concentrations showed ascending trends throughout the study in both yoghurt consuming groups, but changes were not statistically significant.

Comparing the mean differences (T1-T3) among the three groups showed significant decrease for TAC in the conventional yoghurt group compared to the control and significant increase for MDA in the probiotic yoghurt group compared to the control.

The significant changes on blood oxidant and antioxidant parameters are mainly attributed to both the conventional and the probiotic yoghurt groups compared to the control group. Similar to what was observed in lipid profile results, there was not any significant difference between the two yoghurt groups.

There are very few studies specifically on the effect of probiotic yoghurt on antioxidant parameters in humans, and the results are contradictory. In an investigation by Kullisaar et

al (Kullisaar et al, 2003) intake of goat milk enriched with *Lactobacillus fermentum* ME-3, compared to ordinary goat milk, boosted long-term resistance of the lipoproteins to oxidation. It also reduced concentrations of peroxidized lipoproteins, oxidized low density lipoproteins, 8-isoprostanes and glutathione redox ratios. The study showed that the fermented goat milk increased total antioxidative activity.

Kapila et al (Kapila et al, 2006) showed that the TBARS concentrations were lower in the low density lipoprotein fraction of blood serum in rats that were given fermented milk compared to those on a non fat milk diet. These findings show the hypocholesterolemic and antioxidative properties of *Lactobacillus casei ssp. casei*. A study by Fabian and Elmadfa (Fabian and Elmadfa, 2007) demonstrated a meaningful drop in values of total antioxidant capacity of both conventional and *Lactobacillus casei*-enriched yoghurt consuming groups. The study also showed that malondialdehyde and conjugated dienes values in both groups increased. Although many other parameters were altered considerably as a result of the investigation, there were no meaningful differences between the conventional and probiotic groups.

The results from the study by Fabian and Elmadfa (Fabian and Elmadfa, 2007) are somehow similar to the present study. In the present study, the observed effects were seen mainly in both yoghurt consuming groups for TAC concentration. Even though the mean difference (T1-T3) comparison showed significant results for MDA and TAC, in conventional and probiotic yoghurt consuming groups, respectively, those changes were significant only compared to the control group and not compared to the other yoghurt group. The alterations and trends observed in the antioxidant parameter and lipid peroxidation parameters indicate an increase in oxidative stress which could be due to the immune system stimulation caused by administration of both yoghurts.

It has been demonstrated that intake of yoghurt and consumption of lactic acid bacteria stimulate the immune system. For years it was believed that yoghurt had properties that boosted the humans' defense mechanisms against harmful elements. Yet, the specific components in yoghurt that were behind this action were not quite identified. It is suggested that yoghurt's stimulating effect on the immune system come from the bacteria that live in it, but the mechanism through which this happens has not been quite identified. It is the compounds like peptidoglycan, teichoic acid, and polysaccharide in the walls of lactic acid

bacteria cells that have been demonstrated in various studies to be responsible for this action (Takahashi, et al 1993). Live or active lactic acid bacteria particles could stimulate both specific and nonspecific immune response. The immune stimulating activity of the bacteria in the intestine is to a large extent contingent upon their interaction with lymphoid cells in the lumen. As they are readily removed from the mucosa, the nonviable bacteria are supposedly a lot less effective antigens (Muscettola et al, 1994). A number of investigations, though, have demonstrated no difference, between live and dead in terms of their immunogenicity (Hatcher and Lambrecht, 1993).

One of the key elements of peptidoglycan in cell of bacteria is Muramyl dipeptide (MDP). It is important in discharging IL-1. MDP does that through stimulation of Marophages. IL-1 is necessary for production of IFN- γ by lymphocytes. It was shown by Tufano et al (Tufano et al, 1991) that production of interlukins (IL-1, IL-4, IL-6) and TNF- α and IFN- γ are induced by MDP. In the case of interlukins MDP does this through monocytes and in the case IL-4 and IFN- γ through lymphocytes. (Meydani and Ha, 2000)

It should be mentioned, however, that other elements exist in the fermented milk or milk itself such as free fatty acids or peptides could stimulate the immune system, when yoghurt is consumed. These elements are not necessarily the bacterial part of the milk or FM, but they could be the parts which contain vitamins, minerals such as calcium, and proteins and are able to stimulate immune system.

Conjugated linoleic acid (CLA), which is a linoleic acid derivative, can be found in higher amounts in yoghurt compared to the milk source of the yoghurt itself. Products obtained from mammals such as cattle, sheep and goat are the main contributors to the CLA in our food. It has been shown that a correlation exists between CLA in fat tissue and human milk on one hand and fat content in milk and its products on the other. (Jiang et al, 1999; Park et al, 1999)

Increases in the amount of CLA in human adipose tissue were demonstrated to be linked to higher consumption levels of dairy fat (Jiang et al, 1999). This was also the case for increased CLA levels in human milk (Park et al, 1999). CLA was stated to have the ability to stimulate the immune system and also to have anticarcinogenic qualities (Badinga and Greene, 2006). Certain CLA isomers have the capability to stop the manifestation of cyclins. In a study on cancer cells of the breast and colon, Kemp et al (Kemp et al, 2003)

demonstrated that this ability of CLA isomers can be the reason for the anticarcinogenic qualities of CLA.

In research done on humans, it has been demonstrated that intake of yoghurt enhanced activity of phagocytes and NK cell and production of cytokine and antibody. In vitro studies on cells showed similar results, after exposure to lactic acid bacteria (Solis-Pereyra and Lemonnier, 1993; Aattouri and Lemonnier, 1997; Galdeano et al, 2007; Peng et al, 2007; Schifffrin et al, 1995). In a study by Perdigon et al on mice, it was demonstrated that feeding yoghurt and *L. acidophilus* and *L. casei* increased sIgA and sIgA-producing cells in the animals' small intestine (Perdigon et al, 1995); The greater the dosage, the greater the effect. Puri et al. showed in their study that plasma IgA levels in mice that were given yoghurt were considerably higher than those in mice that were given milk. They suggested that the IgA that the B cells in the intestine produce gets into the blood and elevates plasma IgA levels (Puri et al, 1996).

Administering *L. casei* by mouth had similar effects to that of probiotic yoghurt. It caused more sIgA to be available in the lumen. Perdigon et al. (Perdigon et al, 1991) suggested that this increase in sIgA level is a result of lactic acid bacteria's effect on Peyers' patches, which alter the ratios of CD4 and CD8. Meyer et al showed in their investigation (Meyer et al, 2007) that consumption of *Lactobacillus casei*-enriched and conventional yoghurts led to an increase in $\text{TNF-}\alpha$. The normal yoghurt consuming group showed a considerably higher amount of interleukin (IL)-1beta, while the probiotic consuming group showed higher $\text{IFN-}\gamma$. Intake of both yoghurt types boosted pro-inflammatory cytokine production.

Matsuzaki and Chin found in their study that eating *Lactobacillus casei* strain *Shirota* boosts innate immunity through the activity of natural killer cells (Matsuzaki and Chin, 2000). In their study on effects of probiotic supplements on healthy individuals, Christensen et al did not find any positive effect on immune response of their subjects (Christensen et al, 2006). This was true even when high doses of *B. animalis ssp. lactis BB-12* and *L. paracasei ssp. paracasei* were used.

In a study, rats were fed *L. acidophilus* and *L. casei*-enriched *Dahi*. Peroxidation of lipids and formation of NO were reduced as a result. The probiotic *Dahi* preserved antioxidant compounds, like glutathione, and SOD, catalase and GPX effects (Yadav et al, 2008).

Consumption of *Dahi* led to reduction in total cholesterol and blood pressure in humans. It also resulted in a considerable increase in macrophage function. The results also demonstrated the unfavorable effect of cholesterol on the body's immune function (Pawan et al, 2007).

Eating fermented yoghurt enriched with *Lactobacillus acidophilus* and *Bifidobacterium animalis subsp. lacti*, for five weeks resulted in meaningful increase in phagocyte activity as compared with a group that was fed placebo. The two groups showed no difference, however, in oxidative or immune functions (Klein et al, 2008).

Studies done on both humans and animals over the years demonstrate that intake of yoghurt is able to stimulate specific in vitro immune responses, like creation of cytokine, macrophage and lymph cell response. The effect of lactic acid bacteria varies according to each strain's survival potential in the gastro intestinal tract, its ability to function in the acid environment of the gut, and its success in attaching to the intestinal mucosa.

In the present study changes in the oxidative parameters are perhaps caused by immune system stimulation functions of both types of yoghurt. However, cytokine production, phagocyte activity, antibody production, or other immunostimulating activities of yoghurt products were not investigated in this study.

7 CONCLUSION AND SUGGESTIONS

The present study did not show any significant differences in lipid profiles within any group. The mean differences (T1-T3) between the yoghurt consuming groups and the control group showed a decrease in total cholesterol in both yoghurt groups, a decrease in total/HDL cholesterol ratio in both yoghurt groups, and an increase in HDL cholesterol in probiotic yoghurt group compared to the control.

Changes in lipid profiles were observed in both probiotic and conventional yoghurt consuming groups compared to the control. We did not observe significant differences between lipid profile parameters in the two yoghurt groups. The fact that changes in lipid profiles were observed in both yoghurt consuming groups compared to the control group and that there were no differences between the two yoghurt groups, leads us to draw the conclusion that any hypocholesteromic effect might be due to the consumption of fermented milk and not necessarily the probiotic yoghurt. Both products had a positive effect on the lipid profiles of the subjects.

Total antioxidant capacity was significantly lowered throughout the study in both conventional and probiotic yoghurt groups compared to the control group. Mean differences showed a significant decrease in TAC concentration for conventional yoghurt and an increase in MDA in the probiotic yoghurt group. The decrease in antioxidant and the increase in oxidant parameters indicate that the oxidative stress is probably the consequence of stimulating the immune system caused by the administration of yoghurt –probiotic or conventional. For these parameters as well, the observed effects are due to consumption of fermented milk.

Suggestions for future studies:

- To collect fecal samples in order to determine whether the ingested bacteria could also be found in the fecal samples. By measuring the fecal lactoflora, the effects of consuming the lactic acid bacteria on intestinal microflora could be assessed.

- To measure the short chain fatty acids in the feces to assess the effect of lactic acid bacteria on production of the SCFA and to use it as a tool to evaluate the compliance.
- To measure the effects of lactic acid bacteria on factors related to inflammation and immune system, such as interleukins, tumor necrosis factor- α , interferon gamma, CRP and other relevant factor.
- To measure the effects of lactic acid bacteria on the activity of other antioxidant factors, enzymic and non-enzymic antioxidants parameters.
- To study the effects of different strains of lactic acid bacteria on lipid profile and oxidative stress.
- To conduct similar studies and evaluate the effects of long term administration of probiotic bacteria.

SUMMARY

The effect of probiotic yoghurt on plasma lipids has been investigated in a number of studies, but the results are not conclusive. Furthermore, there are limited studies on the effects of probiotic yoghurt on oxidative stress in humans. The goal of this study was to compare the effects of probiotic yoghurt and conventional yoghurt on the lipid profiles and potentially oxidant and antioxidant parameters in healthy women.

In a randomized trial, ninety females between 19-49 years old were allocated into three groups. The first two groups consumed either 300g/day probiotic yoghurt or conventional yoghurt for 6 weeks. The third group did not consume any yoghurt. Both conventional and probiotic yoghurts contained *S. thermophilus* and *L. bulgaricus*. The probiotic yoghurt was further enriched with cultures of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium* BB-12. Fasting blood samples, anthropometric measurements, and a three day dietary record were obtained at the baseline (T1), at the end of the 3rd week (T2), and at the end of the 6th week (T3). Lipid profile parameters were determined by enzymatic methods. Apolipoprotein A and B were analyzed by Immunoturbidometry method. Total antioxidant capacity and malondialdehyde were measured by spectrophotometric method, and oxidized LDL with Eliza.

Results showed that there were no statistically significant differences in height, weight, BMI, age and nutrient intakes among the three study groups at the beginning of the study, and that there were no statistically significant changes throughout the study within any group.

There were no significant changes in the lipid profile within any group throughout the study. Comparing mean differences (T1-T3) between yoghurt consuming groups and the control showed the following: no difference in triglyceride, LDL cholesterol, Apo A and B; decrease in cholesterol in both conventional ($P=0.014$) and probiotic yoghurt groups ($P=0.002$); decrease in total/HDL cholesterol ratios for both conventional ($P=0.006$) and probiotic yoghurt groups ($P=0.000$); increase in HDL cholesterol in probiotic yoghurt group ($P=0.009$).

TAC concentration was significantly lower in the third week (T2) and sixth week (T3) of the study, in both conventional ($T2<T1$; $P<0.01$ & $T3<T1$; $P<0.0001$) and probiotic yoghurt groups ($T2<T1$; $P<0.05$ & $T3<T1$; $P<0.0001$) compared to the baseline values. There were no statistically significant differences in oxidized LDL and MDA among the three intervals of the study within any group. Comparing the mean differences (T1-T3) between yoghurt

consuming groups and the control showed significant decrease in TAC values for the conventional yoghurt group ($P=0.002$), an increase in MDA for the probiotic group ($P=0.038$), and no difference for oxidized LDL.

This study showed that both products had a positive effect on the lipid profile and both yoghurts stimulated the immune system.

ZUSAMMENFASSUNG

Die cholesterinsenkende Wirkung von probiotischem Joghurt wurde bereits in zahlreichen Studien untersucht und kontrovers diskutiert. Darüber hinaus haben sich bislang nur wenige Untersuchungen gezielt mit dem Einfluss von probiotischem Joghurt auf den oxidativen Stress befasst.

Ziel der vorliegenden Studie war es daher, die Wirkung von probiotischem Joghurt auf das Lipidprofil und Parameter des oxidativen Stresses bei gesunden jungen Frauen zu erfassen. An der randomisierten Studie nahmen insgesamt 90 freiwillige Frauen im Alter von 19 bis 49 Jahren teil, welche in drei Gruppen zu je 30 Probanden aufgeteilt wurden. Die Probanden erhielten über sechs Wochen täglich 300g konventionelles, 300g probiotisches oder kein Joghurt (Kontrollgruppe). Beide getesteten Joghurts beinhalteten *S. thermophilus* und *L. bulgaricus*, das probiotischen Produkt enthielt zusätzlich eine Mischkultur bestehend aus *L. acidophilus*, LA-5 und *Bifidobacterium BB-12*. Anthropometrische Messungen und ein 3-Tage-Ernährungsprotokoll wurden durchgeführt, Nüchternblutproben zu Beginn der Studie (T1), drei (T2) und sechs Wochen (T3) nach regelmäßiger Joghurtaufnahme gesammelt.

Sämtliche Blutlipidparameter wurden enzymatisch, Apolipoprotein A und B mittels Immunoturbidometrie bestimmt. Die gesamte antioxidative Kapazität (TAC) und Malondialdehyd (MDA) wurden spektrophotometrisch, oxidiertes LDL mittels ELISA analysiert. Hinsichtlich Alter, Körpergewicht/-größe bzw. BMI und der durchschnittlichen Nährstoffaufnahme zeigten sich weder zwischen noch innerhalb der Gruppen im Studienverlauf signifikante Unterschiede.

In Bezug auf das Lipidprofil konnte im gesamten Studienverlauf in keiner der untersuchten Gruppen eine signifikante Veränderung erfasst werden. Ein Vergleich der Differenzen der Ergebnisse (ΔT) der drei Gruppen ließ nach 6-wöchigem Joghurtverzehr (T1-T3) keine nennenswerten Unterschiede der Konzentration an Triglyzeriden, LDL-Cholesterin, Apo A und B erkennen; Gesamtcholesterin nahm sowohl in der probiotischen ($P=0.002$) als auch in der konventionellen ($P=0.014$) Gruppe signifikant ab. Weiters konnte im Zeitraum T1-T3 eine signifikante Reduktion des Gesamt-/HDL-Cholesterin Quotienten in beiden Gruppen (probiotisch: $P=0.000$; konventionell: $P=0.006$) beobachtet werden. Die Differenz der Plasmakonzentration an HDL-Cholesterin nahm im Untersuchungszeitraum (T1-T3) lediglich innerhalb der probiotischen Gruppe signifikant zu ($P=0.009$).

Verglichen mit den Ausgangswerten waren die Plasmalevel der TAC bereits nach 3- sowie nach 6-wöchigem regelmäßigen Joghurtkonsum sowohl in der probiotischen ($T_2 < T_1$; $P < 0.05$ & $T_3 < T_1$; $P < 0.001$) als auch in der konventionellen ($T_2 < T_1$; $P < 0.01$ & $T_3 < T_1$; $P < 0.001$) Gruppe signifikant erniedrigt. Hinsichtlich der Plasmakonzentrationen an oxidiertem LDL und MDA konnte in keiner der Gruppen eine nennenswerte Veränderung während des Studienzeitraums eruiert werden. Ein Vergleich der Differenzen (ΔT) dieser Parameter (im Zeitintervall T_1 - T_3) zwischen den Interventions- und der Kontrollgruppe ließ eine signifikante Abnahme der TAC-Werte ($p = 0.002$) im Kollektiv, welches konventionelles Joghurt verzehrt hatte, eine signifikante ($P = 0.038$) Erhöhung der MDA-Konzentration in der probiotischen Gruppe, jedoch keine nennenswerten Differenzen in Bezug auf oxidiertes LD erkennen.

Die Studie zeigte, dass konventionelles sowie probiotisches Joghurt einen positiven Einfluss auf das Lipidprofil hat.

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APPENDICES

Nutrition & Biochemistry Department
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**The Effects of Consumption of Probiotic Yogurt on Lipid Profile and
Oxidative Stress Factors in Women
Entry Form**

Name: _____ Surname: _____
Address: _____
Phone number: _____

1. Date of Birth/...../..... Age:
2. Do you suffer from any of the following illnesses?
 - ☐ Diabetes
 - ☐ kidney or liver diseases
 - ☐ Thyroid disorders
 - ☐ Inflammatory intestinal diseases
 - ☐ Diarrhea, colitis
 - ☐ history of heart disease/ stroke
 - ☐ Lactose intolerance
 - ☐ Hypercholesterolemia
 - ☐ Immunodeficiency diseases
 - ☐ Cancer
3. Do you suffer from any other illness?
No Yes (please state:)
4. Have you taken antibiotics in the past month?
No Yes
5. Do you take lipid lowering drugs?
No Yes (please state:)
6. Do you take multi-vitamin or minerals?
No Yes (please state type and dosage:)
7. Do you take any other supplements (Example: omega-3, Vitamin E, fiber)?
No Yes (please state type and dosage:)
8. Do you take a particular medication on a regular basis (Example: Anticoagulants, immuno-suppressant, corticosteroids)?
No Yes (please state type and dosage:)
9. Have you taken any particular drug in the last month?
No Yes (please state type:)
10. Have you used any probiotic products during the past month?
No Yes (please state type:)
11. Do you take hormones?
No Yes (please state type and dosage:)
12. Are you a professional athlete?
No Yes

13. Are you pregnant or breast feeding?
No Yes
14. Do you intend to get pregnant in the following two months?
No Yes
15. Do you smoke?
No Yes (how many per day:)
16. Do have regular menstruations?
No Yes
17. Do you follow any particular diet (vegetarian, vegan...)?
No Yes (state:)
-
18. Weight (kg):
19. Height (cm):
20. Body Mass Index (BMI):
21. Waist (cm):
-
22. Blood pressure (mm/Hg):
-
23. Cholesterol (mg/dl):
24. Triglyceride (mg/dl):
25. CBC results:
- | | | |
|---------------------------------|---------------------------------------|--|
| <input type="radio"/> WBC ----- | <input type="radio"/> MCH ----- | <input type="radio"/> Lymphocyte---- |
| <input type="radio"/> RBD ----- | <input type="radio"/> MCHC ----- | <input type="radio"/> Monocyte ----- |
| <input type="radio"/> Hg ----- | <input type="radio"/> Platelet ----- | <input type="radio"/> Eosinophil ----- |
| <input type="radio"/> HCT ----- | <input type="radio"/> Neutrophil----- | <input type="radio"/> Band cell ----- |
-
26. Occupation:
- Student
 - Employed (please state where: -----)
 - Housewife
27. Education
- High school diploma
 - Bachelors degree
 - Masters and above
28. Do you exercise or engage in physical activity on a regular basis?
- I don't exercise at all
 - Yes I exercise on a regular basis (how many times a week and for how long?).....
29. Do you eat yogurt regularly?(how many times/week)

**The Effects of Consumption of Probiotic Yogurt on Lipid Profile and
Oxidative Stress Factors in Women
Questionnaire 1**

Name: Surname:
Address:
Phone number: Cell phone number:

-
1. Individual Identification code:
 2. Intervention code: 1) Plain top yoghurt 2) colored top yoghurt 3) No yoghurt
-
3. Phase of study (1, 2, 3):
 4. Date of survey:
 5. Date of intervention (receiving first product):
-

6. Weight (kg):
 7. Height (cm):
 8. Body mass index (BMI):
 9. Waist (cm):
-

10. Blood pressure (mm/hg):
-

11. Has your physical activity changed during the past three weeks?
 - a. No
 - b. Yes (explain):
12. Has your diet changed during the past three weeks?
 - a. No
 - b. Yes (explain):
13. Have you suffered from any particular illness during the past three weeks?
 - a. No
 - b. Yes (explain):
14. Did you use any drugs, multi-vitamins or supplements during the past three weeks?
 - a. No
 - b. Yes (explain):
15. Did you follow the instruction regarding the preserving and consumption of the products given to you?
 - a. No (explain):
 - b. Yes

Comments: (date of receiving products and label type)

1. Date:label type:
2. Date:label type:
3. Date:label type:

Date of next visit... /... /...

Interviewed by:

**The Effects of Consumption of Probiotic Yogurt on Lipid Profile and
Oxidative Stress Factors in Women
Questionnaire 2**

Name: Surname:
Address:
Phone number: Cell phone number:

1. Individual Identification code:
2. Intervention code: 1) Plain top yoghurt 2) colored top yoghurt 3) No yoghurt

3. Phase of study (1, 2, 3):
4. Date of survey:

5. Weight (kg):
6. Height (cm):
7. Body mass index (BMI):
8. Waist (cm):

9. Blood pressure (mm/hg):

10. Has your physical activity changed during the past three weeks?
a. No
b. Yes (explain):

11. Has your diet changed during the past three weeks?
a. No
b. Yes (explain):

12. Have you suffered from any particular illness during the past three weeks?
a. No
b. Yes (explain):

13. Did you use any drugs, multi-vitamins or supplements during the past three weeks?
a. No
b. Yes (explain):

14. Did you follow the instruction regarding the preserving and consumption of the products given to you?
a. No (explain):
b. Yes

Comments: (date of receiving products and label type)

4. Date:label type:
5. Date:label type:
6. Date:label type:

Date of next visit: ... / ... / ...

Interviewed by:

CURRICULUM VITAE

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EDUCATION

2006-present: Ph.D. student at the Department of Nutrition Sciences, University of Vienna
1990-1992: Master in Public Health (MPH), University of California at Berkeley, U.S.A.
1981-1986: B.S. in Nutritional Sciences, National Nutrition and Food Technology Research Institute of Iran.

POSITION HELD

1994- present: Instructor at the Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences (TUMS)

PROFESSIONAL EXPERIENCE

1994-2006 **Teaching**
Tehran University of Medical Science
▪Undergraduate Courses: *Basic Nutrition and Diet Therapy, Basic Nutrition*
▪Graduate Courses: *Research Methodology in Health Systems, Nutrition Fieldwork, and Nutrition*

1996-2005 **Workshops**
▪Primary Health Care Systems (PHC); Ministry of health, Tehran

- Prevention and Control of Iron Deficiency Anemia; Ministry of health, Tehran
- Growth Monitoring; Ministry of health, Tehran
- Athlete's Nutrition; TUMS

Thesis Consultant

1998-2006 20 MS. theses in nutrition, TUMS

Research

Administrator of research projects at TUMS:

2006-2009 Effects of Consumption of Probiotic Yoghurt on Lipid Profile and Some Anti-oxidative and Inflammatory Factors in Women

2003-2005 The Prevalence of Obesity and Overweight and Some Associated Factors Among the Kerman High School Girls

Co-administrator at research projects at TUMS:

2003-2004 The Assessment of Anti-oxidant Vitamins of A, E, C, and Malondialdehyde in the Plasma of Women with Breast Cancer, Who are Referred to Cancer Institute of Emam Hospital,

2001-2004 The Prevalence of Obesity and some Associated Factors among Primary School-age Children at Kerman, Kazeroon, Oroomieh, Abadan, and Yazd, Provinces of Iran,

2000-2002 The Prevalence of Riboflavin Deficiency and its Relation with Vitamin A and Iron Status among School-aged Children in Villages of the Province of Kerman,

1999-2002 Effects of Nutrition Education on Nutritional Status of School-aged Girls (11 to 14) in the City of Kerman,

1997-1999 A Study of Planning and Administering Models for Food and Nutrition Security,

1995-1999 Ways to Improve Nutritional Status of Peri-urban Settlers of the City of Kerman,

Services

2000-2001 Nutrition counseling at Shariati Hospital, Tehran

HONORS

1991-1992 Research grant, University of California, Berkeley, School of Public Health. U.S.A.

PROFESSIONAL SERVICES

2004-2007 Vice administrator, Nutrition Education Training Program in Iran; World Bank Program

1997-2006 Member, Board of Directors for Research and Education, (INS)

1995-2006 Member, Committee for Planning and Coordination of Iranian Nutritional Congresses (INS)

1995-1997 Member, Committee for Community-Oriented Nutrition Education (CONE)

1995 Member, Organizing Committee (Head of the Public Relations), of the Forth Iranian Nutrition Congress

MEMBERSHIP

1994-present Iranian Nutrition Society (INS)

LANGUAGES

Persian, English

PUBLICATIONS IN ENGLISH

Abstracts and Posters Presentations in International Conferences

- 1 **Sadrzadeh-Yeganeh H**, Angoorany P, Keshavarz SA, Rahimi A, Ahmady B. Comparison of the effectiveness of two nutritional education methods concerning breakfast on practice of fourth grade girls at Tehran's 6th educational and training district. *The 1st World Public Health Nutrition Congress*, Barcelona, Spain. 2006
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- 4 Jarollahi H, **Sadrzadeh Yeganeh H**, Nazemi L, Alavi A, Afkhami A, Jafaryrad S, Sahargahy B. A rapid survey to study the effect of physical activity and energy intake on the percentage of body fat, WHR, and BMI in 19-49 year old women in a village in Chatroud in the province of Kerman, Iran. *The 5th International Conference on Nutrition and Fitness*: Athens, Greece. 2004

- 5 Keshavarz S, Hozoori M, Hosseini M, **Sadrzadeh Yeganeh H**, Sabour AA. Effect of two meal with different carbohydrate to protein ratio on performance and serum level of insulin and glucose in male soccer after exercise, Tehran, Iran. *The 5th International Conference on Nutrition and Fitness*: Athens, Greece. 2004

- 6 **Sadrzadeh Yeganeh H**. Rapid survey of the relation between energy and macronutrients intake, on the one hand, and BMI, WHR, and percentage of body fat of the elderly, on the other, at one of the health centers in the province of Kerman, Iran. *Asian Congress of Nutrition*, New Delhi, India. 2003

- 7 Siassi F, Harrison G, Keighobadi K, Pouransari Z, Jarollahi H, **Sadrzadeh Yeganeh H**, et al. Prevalence and severity of riboflavin, vitamin A and iron deficiencies in rural school children in Kerman province of Iran. *Annals of Nutrition & Metabolism*, 2001; 45(suppl 1) 01.

- 8 **Sadrzadeh Yeganeh H**, Siassi F, Keyghobadi K, et al. The study of effects of nutrition education on knowledge and practice of mothers, and its relation with birth weight in squatters settlements of kerman. *Annals of Nutrition & Metabolism*, 2001; 45(suppl 1) 01.

Translations

Ghasemi H, *Nutrition Relevant Policies in the Health Sector, Planning and Administration*, Tehran:UNICEF, 1997

PUBLICATIONS IN PERSIAN

Books

Nutrition for Children at Primary School Age, co-authored with H. Pishva, Tehran;Ghasidehsara, 2002. 140 pages

Journals Articles

- 1 Angoorany P, Keshavarz SA, Rahimi A, **Sadrzadeh-Yeganeh H**. The Effect of a Nutritional Teaching Booklet Concerning Breakfast on the Knowledge of Fourth Grade Girls at Tehran's Sixth Educational and Teaching District. *Tehran University Medical Journal*, 2007; 65(suppl 2): 49-54
- 2 **Sadrzadeh-Yeganeh H**, Alavi AMH, Dorosty-motlagh AR, Mahmoodi M, Jarollahi N, Chamari M. Relationship between Obesity and some Nutritional Behavior among High-school Girls in Kerman. *Journal of the Iranian Institute for Health Sciences Research*, 2007; 6(3): 193-199
- 3 Shahrabi-farahani J, Dorosty-motlagh AR, Djalali M, **Sadrzadeh-Yeganeh H**, Farvid MS. The Effect of Two-week Supplementation with Ascorbic Acid on Blood Leads Level of Welders. *Journal of Rafsanjan University of Medical Sciences*, 2006, 5(2): 75-84
- 4 Aghvami T, Keshavarz A, Sadeghi MR, Zeraati H, **Sadrzadeh-Yeganeh H**, Negahdar M. Plasma Antioxidant Vitamin Levels and Lipid Peroxidation in Breast Cancer Patients. *Iranian Journal of Public Health*. 2006; 35(1): 42-47
- 5 Hoozori M, Keshavarz A, Hosseini M, **Sadrzadeh-Yeganeh H**, Sabour AA, Farajzade SH. Effect of Two Different Regimes of Carbohydrate and Protein on Performance and Serum Level of Insulin and Glucose in Soccer Players, *Tehran University Medical Journal*, 2006; 64(4): 26-36
- 6 **Sadrzadeh-Yeganeh H**, Angoorany P, Keshavarz SA, Rahimi A, Ahmady B. Comparison of the Effectiveness of Two Nutritional Education Methods Concerning Breakfast on Practice of Fourth Grade Girls at Tehran's 6th Educational and Training District. *Journal of School of Public Health & Institute of Public Health Research*. 2005, 4 (1): 65-72
- 7 Jafarirad S, Keshavarz SA, **Sadrzadeh-Yeganeh H**, Dorosti A. Assessment of Anthropometric Status and Nutrients Intake in Adolescent Girls in Mazandaran Province, Iran. *Journal of the Iranian Institute for Health Sciences Research*, 2005; 4(3): 217-223.
- 8 Nadjarzadeh A, Siassi F, Harrison G, Pouransari Z, Djallali M, Jarollahi H, **Sadrzadeh Yeganeh H**, Alavi Naeeni AM. Prevalence of Anemia and Iron Deficiency and Their Association with Economic Status Among Primary School Children in Rural Areas of Kerman Province. *Iranian Journal of Nutrition*. 2005; 1(1) p: 28-32.
- 9 Ghavasseh M, Keshavarz SA, Sotoudeh G, **Sadrzadeh Yeganeh H**, Rahimi A, Khadjeh-Nassiri F. A Study of Gestational Nutrition education Effect on Nutritional Knowledge, Attitude, and Practice of Pregnant Women Under Cover of Urban Health Center of Jahrom. *Iranian Journal of Nutrition*. 2005; 1(1) p: 38-43.

- 10 Doost-Mohammadian A, keshavrz AR, Dorosty M, Mahmoudi M, **Sadrzadeh-Yeganeh H**. Survey of the Nutritional Status and Relationship Between Physical Activity and Nutritional Attitude with Index of BMI-for-Age in Semnan Girl Secondary School. *Journal of Semnan University of Medical Sciences*, 2005;6(3):187-194.
- 11 Taslimi Taleghani M, Djazayeri A, Keshavarz SA, **Sadrzadeh Yeganeh H**, Rahimi A. The effect of Soci-economic Status on Effectiveness of Nutrition Education in Increasing Knowledge and Attitude of Girls in First-Year of Middle School in Tehran, *Journal of Faculty of Medicine*, 2005; 63(1): 77-62
- 12 Taslimi Taleghani M, Djazayeri A, Keshavarz SA, **Sadrzadeh Yeganeh H**, Rahimi A. Comparison of the Effectiveness of Two Nutrition Education Methods on the Nutritional Knowledge, Attitude and Practice of First Grade School Girl Students in Tehran. *Journal of School of Public Health & Institute of Public Health Research*, 2004; 2 (4) p: 69-70
- 13 Keighobadi K, Siassi F, Malekafzali H, Jarollahi N, **Sadrzadeh Yeganeh H**, et al . Impacts of mother's education on the nutritional status of children among households living in outskirts of Kerman, *Hakim Research Journal*, 2002;5(1):p.49-57.

Poster presentations in congresses (Abstracts)

- 1 Effects of individual factors on obesity among high school girls in Kerman. *Abstracts, 10th Nutrition Congress*, Tehran, Iran, 2008.
- 2 Dietary factors associated with nutritional status in female adolescents, semnan. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 3 BMI, energy and nutrient intake among Semnan's high school girls. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 4 Dietary and anthropometric status of nursing home residents in Tehran and Shemiranat *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 5 Influence of vitamin C supplementation on lead and Hemoglobin levels among welder workers. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 6 Relationship between nutritional behavior and obesity among high-school girls in Kerman. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 7 Demographic and socio-economic factors in relation to nutritional status in female teenagers in Semnan. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 8 Maternal nutritional KAP of 6-36 month-old children, Kerman. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.

- 9 Nutritional status assessment and its related factors in 6-36 month-old children, Kerman. *Abstracts, 9th Nutrition Congress*, Tehran, Iran, 2006.
- 10 A rapid study of association between energy and macro-nutrients with BMI and WHR and in elderly population of the health centers in the province of Kerman, Iran. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 11 A rapid study of effect of individual and socio-economic factors on BMI and WHR and percentage of body fat in elderly population of the health centers in the province of Kerman, Iran. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 12 A rapid study of percentage of body fat in 19-49 years old women of the village of Houdk. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 13 A study of prevalence of obesity among the women in Houdk. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 14 A study of the relation between socio-economic factors and education on improvement of nutritional knowledge of first grade school girls in Tehran. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 15 A study of the relation between number of pregnancies and percentage of energy intake among 19-49 years women in Houdk. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 16 Comparison the effect of two different meals after glycogen depletion on the physical performance, insulin level and serum glucose of male football players. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 17 A study of association between metabolic diseases, obesity among family members, season of birth, and medicine intake with obesity among 19 to 49-year-old women of Chatroud. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 18 The effect of some nutrients on iron status of primary school children of the villages of Kerman province. *Abstracts, 8th Nutrition Congress*, Tehran, Iran. 2004.
- 19 A study of riboflavin status of primary school children and its relation with riboflavin, protein, and energy intake. *Abstracts, 8th Nutrition Congress*, Tehran, Iran, 2004.
- 20 Factors affecting anemia and iron status in school-aged children of the Province of Kerman. *Abstracts, 7th Nutrition Congress*, Rasht, Iran, 2002.
- 21 Effects of nutrition education of mothers on anthropometrics indexes of 6 to 47-months children. *Abstracts, 7th Nutrition Congress*, Rasht, Iran, 2002.
- 22 Survey of riboflavin status and of factors affecting it among school-aged children in villages of the province of Kerman. *Abstracts, 7th Nutrition Congress*, Rasht, Iran, 2002.

- 23 Study of effects of mothers' nutrition education on their nutritional knowledge and practice, its relation with children's birth weight in peri-urban areas of the city of Kerman. *Abstracts, 6th Nutrition Congress*, Ahvaz, Iran, 2001.
- 24 Study of effects of mothers' nutrition education on riboflavin status of 6 to 47-month children in the peri-urban areas of the city of Kerman. *Abstracts, 6th Nutrition Congress*, Ahvaz, Iran, 2001.
- 25 Study of effects of mothers' nutrition education on their nutritional knowledge, attitude, and practice (KAP), and its relation with protein and energy intake of children in two peri-urban areas of the city of Kerman. *Abstracts, 6th Nutrition Congress*, Ahvaz, Iran, 2001.
- 26 A comparison of ascorbic acid status in 6 to 47-month children of two peri-urban areas of the city of Kerman before and after nutrition education intervention. *Abstracts, 6th Nutrition Congress*, Ahvaz, Iran, 2001.
- 27 Ascorbic acid status of mothers and factors affecting among mothers in peri-urban areas of the city of Kerman. *Abstracts, 5th Nutrition Congress*, Tehran, Iran, 1999.
- 28 Effects of mothers' nutrition education on nutritional status of children aged 24-35 months and factors affecting it. *Abstracts, 5th Nutrition Congress*, Tehran, Iran, 1999.
- 29 A Study of the relation between mothers' nutritional KAP and nutritional and growth status of 6 to 60-month children in the peri-urban areas of the city of Kerman. *Abstracts, 4th Nutrition Congress*, Tehran, Iran, 1997.
- 30 Study of Riboflavin status and of factors affecting it among 6 to 60-month children in the peri-urban areas of the city of Kerman". *Abstracts, 4th Nutrition Congress*, Tehran, Iran, 1997.
- 31 A Study of the relation between mothers' nutritional practice during pregnancy and birth weight in the peri-urban areas of the city of Kerman. *Abstracts, 4th Nutrition Congress*, Tehran, Iran, 1997.
- 32 A Study of the relation between mothers' nutritional KAP and socio-economic status in the peri-urban areas of the city of Kerman". *Abstracts, 4th Nutrition Congress*, Tehran, Iran, 1997.

